

KIDNEY TRANSPLANTATION IN THE IMMUNOLOGICALLY HIGH-RISK
PATIENT

by
Babak John Orandi, MD MSc

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ABSTRACT

Survival for dialysis patients is dismal. They have an adjusted mortality rate 6.5-7.9 times higher than the general population. Kidney transplant (KT) recipients enjoy significant survival and quality of life advantages compared to remaining dialysis-dependent (1-5). Unfortunately, kidney demand far exceeds supply, with over 90,000 on the wait list (5). Highly sensitized patients constitute an increasingly large part of the wait list (6).

Advancements in desensitization have allowed for KT across previously insurmountable immunological barriers; however, incompatible live donor kidney transplantation (ILDKT) is in its nascency and the literature is limited by single-center data, small sample sizes, and publication bias. ILDKT risks are generally considered to be higher than for compatible KT, but these risks have never been quantified precisely. Chapter 2 does precisely this using primary data collected from 22 U.S. transplant centers, constituting the largest cohort of ILDKT patients in existence.

ILDKT risks are not limited to recipients. The federal government provides strict oversight of transplant outcomes. Chapter 2 quantifies the regulatory risk centers assume when they transplant immunologically high-risk patients.

IKT patients are also at elevated risk of antibody-mediated rejection (AMR), which mediates much of the graft loss in ILDKT. Chapter 3 details the formation of the world's largest cohort of AMR patients, defined using strict clinical, pathological, and

immunologic criteria, and quantification of the risk of graft loss associated with AMR by transplant type.

There exists an uncommon, but virulent phenotype of AMR in ILDKT patients that is rapid in onset, severe in the graft dysfunction it causes, difficult to treat, and immediately graft-threatening without prompt action. Chapter 4 describes these patients in detail and compares the early rescue rate and impact of the severe AMR episode on the development of transplant glomerulopathy between salvage modalities, offering novel insight into the management of this challenging and devastating ILDKT complication.

Overall, this dissertation explores the risk of ILDKT to patients and centers, and delves into particular aspects of AMR within the context of ILDKT.

Academic Advisor

Dorry L. Segev, MD PhD

Research Advisor

Robert A. Montgomery, MD DPhil

Thesis Readers

James A. Tonascia, PhD

Jennifer L. Dodson, MD PhD

Niraj M. Desai, MD

O. Joseph Bienvu, MD PhD

Brian S. Caffo, PhD

Robert S. Lawrence, MD

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TABLE OF CONTENTS

TABLE OF CONTENTS	vii
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS.....	x
CHAPTER 1 - Introduction: A Broad Overview of Kidney Transplantation in the Immunologically High-Risk Patient	1
CHAPTER 2 - Quantifying the Risk of Incompatible Kidney Transplantation: A Multi-Center Study	8
CHAPTER 3 - Quantifying Renal Allograft Loss following Early Antibody-Mediated Rejection	31
CHAPTER 4 - Eculizumab and Splenectomy as Salvage Therapy for Severe Antibody-Mediated Rejection Following HLA-Incompatible Kidney Transplantation	53
CHAPTER 5 - Conclusion	93
REFERENCES	87
CURRICULUM VITAE.....	97
BRIEF BIOSKETCH.....	110

LIST OF TABLES

CHAPTER 2 - Quantifying the Risk of Incompatible Kidney Transplantation: A Multi-Center Study	8
Table 1. Characteristics of Transplant Recipients, by Anti-HLA Antibody Strength....	25
Table 2. Adjusted Risk of All-Cause Graft Loss and Mortality in the First Year Post-Transplant and After the First Year By Antibody Strength	26
Table 3. Effect of Incompatible Live Donor Kidney Transplantation on Risk of Flagging by Centers for Medicare & Medicaid Studies in a Simulation.....	27
CHAPTER 3 - Quantifying Renal Allograft Loss following Early Antibody-Mediated Rejection	31
Table 1. Patient Characteristics of the Overall Cohort, the Antibody-Mediated Rejection Group, and the Matched Controls	47
Table 2. Estimates of Death-Censored Graft Survival.....	48
Table 3A. Antibody-Mediated Rejection Risk of Graft Loss, by Transplant Type.....	49
Table 3B. Antibody-Mediated Rejection Incidence and Presentation.....	49
CHAPTER 4 - Eculizumab and Splenectomy as Salvage Therapy for Severe Antibody-Mediated Rejection Following HLA-Incompatible Kidney Transplantation	53
Table 1. Patient Characteristics by Treatment Received.....	73
Table 2. Sensitization, Donor-Specific Antibody Strength, and Treatment of Antibody-Mediated Rejection.....	74

LIST OF FIGURES

CHAPTER 2 - Quantifying the Risk of Incompatible Kidney Transplantation: A Multi-Center Study	8
Figure 1. All-Cause Graft Loss, by Antibody Strength	28
Figure 2. Post-Transplant Mortality, by Antibody Strength	29
Figure 3. The Effect of Incompatible Live Donor Kidney Transplantation on Risk of Flagging by Centers for Medicare & Medicaid Studies in a Simulation.....	30
CHAPTER 3 - Quantifying Renal Allograft Loss following Early Antibody-Mediated Rejection	31
Figure 1. Death-Censored Graft Loss by Development of Antibody-Mediated Rejection	50
Figure 2. Death-Censored Graft Loss, by Development of Subclinical Antibody-Mediated Rejection	51
Figure 3. Death-Censored Graft Loss, by Presentation of Antibody-Mediated Rejection	52
CHAPTER 4 - Eculizumab and Splenectomy as Salvage Therapy for Severe Antibody-Mediated Rejection Following HLA-Incompatible Kidney Transplantation.....	53
Figure 1. Death-censored graft survival by treatment received.....	76
Figure 2. Post-transplant renal function over time by treatment received	77
Figure 3. Serum creatinine, urine output, antibody levels, and details of peri-transplant events in a representative patient from the splenectomy alone group (A), the eculizumab alone group (B), and the combination treatment group (C).	78
Figure 4. Banff chronic glomerulopathy score at the time of antibody-mediated rejection-defining biopsy, 6 months, and 12 months post-transplant by treatment received.....	82
Figure 5. Pathologic findings on the antibody-mediated rejection-defining biopsy and subsequent biopsies in a patient representative of each treatment group.	83

LIST OF ABBREVIATIONS

aHR--adjusted hazard ratio
AMR--antibody-mediated rejection
CDC--complement-dependent cytotoxic crossmatch
cg--chronic glomerulopathy
ci--interstitial fibrosis
CMS--Centers for Medicare & Medicaid Studies
CPRA--calculated panel reactive antibody
ct--tubular atrophy
DSA--donor-specific antibody
ESRD--end stage renal disease
FCXM--flow cytotoxic crossmatch
g--glomerulitis
HCV--hepatitis C virus
HRSA--Health Resources and Services Administration
ILDKT--incompatible kidney transplantation
IQR--interquartile range
IVIg--intravenous immunoglobulin
MFI--mean fluorescence intensity
NA--not available
NT--not tested
OPTN--Organ and Procurement Transplantation Network
PCC--positive cytotoxic crossmatch
PFNC--positive flow, negative cytotoxic crossmatch
PLNF--positive Luminex , negative flow crossmatch
PP--plasmapheresis
PRA--panel reactive antibody
PSR--Program Specific Report
PTC--peritubular capillaries
PTD--post-transplant day
SD--standard deviation
SRTR--Scientific Registry of Transplant Recipients

CHAPTER 1 - Introduction: A Broad Overview of Kidney Transplantation in the Immunologically High-Risk Patient

For patients with end stage renal disease, their survival on dialysis is dismal. Dialysis-dependent patients have an adjusted all-cause mortality rate 6.5-7.9 times higher than the general population (5). Kidney transplant (KT) recipients enjoy significant survival and quality of life advantages compared to remaining dialysis-dependent (1-4), including a reduction in all-cause mortality to 1.0-1.5 times higher than the general population. Unfortunately, the demand for kidneys far exceeds the supply, with over 90,000 on a wait list that grows 4-8% annually (5, 7).

In 1969, cross match testing was introduced, allowing HLA-compatible KT, and thus significantly diminishing the common complication of hyperacute rejection and its concomitant graft loss (8). This allowed transplantation to proceed more safely and with more reliable outcomes. The introduction of more sensitive methods of antibody detection, including flow cytometric crossmatching and the Luminex platform has made compatible KT even safer; however, patients with broad pre-existing sensitivities (from exposure to foreign antigens through previous transplants, pregnancies, or blood transfusions) or hard-to-match blood types have found it much more difficult, and in some cases, impossible, to find a compatible graft, relegating them to a *de facto* permanent position on the wait list. This disparity has widened significantly over time.

Currently, nearly 40% of candidates on the wait list are sensitized (9, 10), prompting many centers, especially those with prolonged wait lists, to aggressively utilize available organs, even suboptimal ones (11). In an effort to ameliorate the supply-demand mismatch, kidney paired donation, the so-called “domino transplants,” were developed to offer hope to incompatible patients (12, 13), though the current volume of paired donations is not enough to help the vast majority of incompatible transplant candidates (14).

Advances in desensitization protocols have allowed for transplantation across antibody barriers that used to be contraindications to transplant (15-18). Incompatible live donor kidney transplantation (ILDKT) is becoming an increasingly utilized approach to treating highly sensitized patients, particularly because of compelling evidence that patients undergoing desensitization and subsequent ILDKT have a two-fold survival benefit compared to remaining on dialysis waiting for a compatible KT (15). Some centers now incorporate kidney-paired donation and desensitization protocols to improve further matching between donors and recipients (18-22).

Desensitization works by eliminating circulating antibody and/or minimizing antibody production. The physical removal of antibody from the circulation can be accomplished through plasmapheresis (PP), plasma exchange, or immunoadsorption (23). Intravenous immunoglobulin (IVIg) functions to abrogate the effects of antibody through a variety of mechanisms, but its most immediate effect is to neutralize circulating antibody (24, 25). The use of complement inhibitors halts the complement-mediated destruction of foreign

cells that have been targeted for destruction by antibody binding (26, 27). The inhibition of antibody production can be achieved through splenectomy, which removes antibody-producing plasma cells and their precursor cells (28). Several pharmacologic agents are also available to prevent antibody production. Rituximab, an anti-CD20 monoclonal antibody, destroys pre-B and mature B lymphocytes before they can become antibody-producing plasma cells (29). Proteasome inhibition is achieved by bortezomib, which induces apoptosis of plasma cells (30, 31).

A number of combinations of these therapies have been used, but the three most widely published protocols are those of the Johns Hopkins Hospital, the Mayo Clinic, and Cedars-Sinai Medical Center. The Hopkins protocol involves every-other-day pre-transplant PP followed by post-PP low-dose IVIg (32). At the initiation of PP and IVIg, patients are also started on tacrolimus and mycophenolate mofetil. At the time of transplant, patients are prescribed induction therapy and steroids. Patients also receive post-transplant PP and IVIg. Alternatively, the Mayo Clinic protocol involves pre-transplant plasma exchange, induction with anti-thymocyte globulin, and maintenance immunosuppression with tacrolimus, prednisone, and mycophenolate mofetil (27). Patients are given 1200 mg of the C5-complement inhibitor eculizumab immediately prior to transplantation, 600 mg on post-operative day 1, and then 600 mg weekly for four weeks. The original Cedars-Sinai protocol involved the administration of monthly infusions of high-dose IVIg (33), though an updated protocol adds a single dose of rituximab (34).

While desensitization and ILDKT allow patients to come off the transplant list and enjoy the survival and quality of life benefits of kidney transplantation (3, 15), it has created a cohort of patients at an unusually high risk of antibody-mediated rejection (AMR), a major threat to long-term graft survival. While unsensitized, compatible recipients can develop *de novo* AMR at a rate of 3-7 percent (35, 36), patients with donor-specific antibody (DSA) prior to transplantation have a 35% risk of developing AMR (35). Blood group incompatible patients have a nearly 10% risk of AMR (37).

AMR, which is the result of the body's humoral arm of the immune system, leads to a number of characteristic histologic changes within the allograft and is primarily directed against the endothelium of the microvasculature (38). Hallmarks of AMR are deposition of complement components C3d/C4d (though recent updates to the Banff Classification of Rejection now allow for C4d-negative AMR (39)), peritubular capillaritis, glomerulitis, thrombotic microangiopathy, interstitial fibrosis and/or hemorrhage, tubular atrophy, and microvascular thrombosis (40). Clinically, these patients often present with decreased urine output, hypertension, pyuria, proteinuria, and an acute rise in serum creatinine--a late finding indicating considerable allograft damage. Occasionally, patients will present with fever and tenderness, pain, or swelling over the graft. The treatment of AMR typically involves the same therapies as desensitization (26, 28, 31, 41-43). Importantly, the development of AMR, even subclinical AMR, is associated with significantly reduced graft survival (44-46).

And while ILDKT has opened the possibility for highly sensitized patients to benefit from KT, a number of significant challenges have thus far limited more widespread adoption of KT. The first limitation relates to the current state of the body of the ILDKT literature. Thus far, all of the studies on ILDKT are single-center studies, most often dominated by a few relatively high-volume centers (15, 27, 34, 47), and therefore subject to the limitations of relatively small sample sizes, publication bias, and significant heterogeneity of patient populations, desensitization and post-transplant management. Other factors limiting more widespread adoption of ILDKT is that it is labor- and resource-intensive, even by transplant center standards. Furthermore, tolerance for risk in the utilization of suboptimal organs varies dramatically across centers (11), and so it stands to reason that many transplant surgeons are reluctant to take on additional risk in the form of ILDKT. This aversion to risk is compounded by the fact that solid organ transplantation is highly regulated by the Centers for Medicare & Medicaid Studies (CMS), with strict scrutiny of graft and patient outcomes using mandatory outcomes benchmarks (48). The risk adjustment models used by CMS do not currently account for ILDKT, meaning that ILDKT outcomes are held to the same standard as compatible KT (49). The current absence of consideration of this factor may create a disincentive for centers to perform ILDKT despite its documented benefits for highly sensitized patients. Indeed, a recent consensus conference on transplant center surveillance methods aptly noted, "Failure to adequately adjust outcomes for risk may cause programs to avoid performing transplants involving suitable but high-risk candidates and donors" (50). There is evidence to suggest that this is the case, which may limit access to transplantation for sensitized patients and lead to disposal of less-than-optimal but still

acceptable organs (51-53). Furthermore, failure to account for high-risk candidates puts transplant centers in jeopardy of punitive regulatory action for what may be satisfactory outcomes when properly and accurately interpreted in light of candidates' true high-risk nature (54).

The field of ILDKT has advanced tremendously in a relatively short amount of time, allowing for the transplantation of kidneys across previously impossible antibody barriers; however, a number of significant challenges to improving access to and outcomes of ILDKT remain. This dissertation seeks to address several of these challenges. ILDKT risks are generally considered to be higher than for compatible KT, but these risks have never been quantified precisely. Chapter 2 does precisely this by using primary data collected from 22 U.S. transplant centers, constituting the largest cohort of ILDKT patients in existence. Chapter 2 also quantifies the regulatory risk transplant centers assume when they transplant immunologically high-risk patients to address some of the regulatory disincentives to ILDKT that are currently in place. Chapter 3 details the formation of the world's largest cohort of AMR patients, defined using strict clinical, pathological, and immunologic criteria, and quantification of the risks of graft loss and death associated with AMR by transplant phenotype. Chapter 4 describes a subset of AMR patients who have a particularly aggressive form of AMR and compares the early rescue rate and impact of the severe AMR episode on the development of transplant glomerulopathy between salvage modalities, offering novel insight into the management of this challenging and devastating ILDKT complication.

CHAPTER 2 - Quantifying the Risk of Incompatible Kidney Transplantation: A Multi-Center Study

Babak J. Orandi¹, Jacqueline M. Garonzik-Wang¹, Allan B. Massie¹, Andrea A. Zachary¹, John R. Montgomery¹, Kyle J. Van Arendonk¹, Mark D. Stegall², Stanley C. Jordan³, Jose Oberholzer⁴, Ty B. Dunn⁵, Lloyd E. Ratner⁶, Sandip Kapur⁷, Ronald P. Pelletier⁸, John P. Roberts⁹, Marc L. Melcher¹⁰, Pooja Singh¹¹, Debra L. Sudan¹², Marc P. Posner¹³, Jose M. El-Amm¹⁴, Ronald Shapiro¹⁵, Matthew Cooper¹⁶, Gregory S. Lipkowitz¹⁷, Michael A. Rees¹⁸, Christopher L. Marsh¹⁹, Bashir R. Sankari²⁰, David A. Gerber²¹, Paul Nelson²², Jason Wellen²³, Adel Bozorgzadeh²⁴, A. Osama Gaber²⁵, Robert A. Montgomery¹, Dorry L. Segev¹.

Author Affiliations: ¹Johns Hopkins Hospital, Departments of Surgery and Medicine; ²Mayo Clinic, Department of Surgery; ³Cedars-Sinai Comprehensive Transplant Center; ⁴University of Illinois-Chicago, Department of Surgery; ⁵University of Minnesota, Department of Surgery; ⁶Columbia University Medical Center, Department of Surgery; ⁷New York Presbyterian/Weill Cornell Medical Center, Department of Surgery; ⁸The Ohio State University, Department of Surgery; ⁹University of California-San Francisco, Department of Surgery; ¹⁰Stanford University Medical Center, Department of Surgery; ¹¹Thomas Jefferson University Hospital, Department of Medicine; ¹²Duke University Medical Center, Department of Surgery; ¹³Virginia Commonwealth University, Department of Surgery; ¹⁴Integrus Baptist Medical Center, Transplant Division; ¹⁵University of Pittsburgh Medical Center, Department of Surgery; ¹⁶Medstar Georgetown Transplant Institute; ¹⁷Baystate Medical Center, Department of Surgery; ¹⁸University of Toledo Medical Center, Department of Urology; ¹⁹Scripps Clinic and Green Hospital, Department of Surgery; ²⁰St. Vincent Hospital, Department of Urology; ²¹University of North Carolina Medical Center, Department of Surgery; ²²St. Luke's Episcopal Hospital, Department of Surgery; ²³Barnes-Jewish Hospital, Department of Surgery; ²⁴University of Massachusetts Memorial Medical Center, Department of Surgery; ²⁵Houston Methodist Hospital, Department of Surgery

ABSTRACT

Incompatible live donor kidney transplantation (ILDKT) offers patients with donor-specific anti-HLA antibody (DSA) a survival advantage over dialysis. Program Specific Reports (PSR) fail to account for ILDKT, placing this practice at regulatory risk. We collected DSA data, categorized as positive Luminex, negative flow (PLNF) (n=185), positive flow, negative cytotoxic (PFNC) (n=536), or positive cytotoxic crossmatch (PCC) (n=304), from 22 centers. We tested associations between DSA, graft loss, and mortality after adjusting for PSR model factors, using 9,669 compatible patients as a comparison. PLNF patients had similar graft loss; however, PFNC (aHR=1.64, 95%CI:1.15-2.23, P=0.007) and PCC (aHR=5.01, 95%CI: 3.71-6.77, P<0.001) were associated with increased graft loss in the first year. PLNF patients had similar mortality; however, PFNC (aHR=2.04; 95%CI: 1.28-3.26; P=0.003) and PCC (aHR=4.59; 95%CI: 2.98-7.07; P<0.001) were associated with increased mortality. We simulated CMS flagging to examine ILDKT's effect on flagging risk. Compared to equal-quality centers performing no ILDKT, centers performing 5%, 10%, or 20% PFNC had a 1.19, 1.33, and 1.73-fold higher odds of flagging. Centers performing 5%, 10%, or 20% PCC had a 2.22, 4.09, and 10.72-fold higher odds. Failure to account for ILDKT's increased risk places centers providing this life-saving treatment in jeopardy for regulatory intervention.

ABSTRACT

Incompatible live donor kidney transplantation (ILDKT) offers patients with donor-specific anti-HLA antibody (DSA) a survival advantage over dialysis. Program Specific Reports (PSR) fail to account for ILDKT, placing this practice at regulatory risk. We collected DSA data, categorized as positive Luminex, negative flow (PLNF) (n=185), positive flow, negative cytotoxic (PFNC) (n=536), or positive cytotoxic crossmatch (PCC) (n=304), from 22 centers. We tested associations between DSA, graft loss, and mortality after adjusting for PSR model factors, using 9,669 compatible patients as a comparison. PLNF patients had similar graft loss; however, PFNC (aHR=1.64, 95%CI:1.15-2.23, P=0.007) and PCC (aHR=5.01, 95%CI: 3.71-6.77, P<0.001) were associated with increased graft loss in the first year. PLNF patients had similar mortality; however, PFNC (aHR=2.04; 95%CI: 1.28-3.26; P=0.003) and PCC (aHR=4.59; 95%CI: 2.98-7.07; P<0.001) were associated with increased mortality. We simulated CMS flagging to examine ILDKT's effect on flagging risk. Compared to equal-quality centers performing no ILDKT, centers performing 5%, 10%, or 20% PFNC had a 1.19, 1.33, and 1.73-fold higher odds of flagging. Centers performing 5%, 10%, or 20% PCC had a 2.22, 4.09, and 10.72-fold higher odds. Failure to account for ILDKT's increased risk places centers providing this life-saving treatment in jeopardy for regulatory intervention.

INTRODUCTION

Patients with anti-HLA donor-specific antibody (DSA) undergoing desensitization and subsequent live donor kidney transplantation (referred to as ILDKT, or incompatible live donor kidney transplantation, for the purposes of this manuscript) enjoy a two-fold survival benefit compared to similar patients who remain on dialysis while waiting for a compatible donor (15). For most highly sensitized patients the choice is not between ILDKT and a compatible (no DSA) transplant. Rather, long waits on dialysis and high mortality rates are the only alternatives to desensitization. Currently, algorithms for calculating center-specific expected outcomes do not consider the survival benefit derived from ILDKT. Furthermore, risk adjustments do not distinguish ILDKT from compatible transplants. Based on a recent survey of U.S. transplant centers, ILDKT have become mainstream procedures, performed at approximately 50-70% of transplant centers nationwide (55).

Single-center reports suggest that ILDKT outcomes are not as good as compatible kidney transplants (56-58). However, differences in outcomes have not been well characterized outside of large volume, more experienced centers. Regulatory organizations currently hold the outcomes of ILDKT patients to the same standard as compatible recipients, thereby potentially penalizing centers that perform these transplants. Better outcome data from ILDKT will inform future decisions about changing risk adjustments to encourage transplant centers to help their patients more fully realize the survival benefits associated with desensitization and ILDKT.

In the development of the Program Specific Reports (PSR), centers are given "credit" for transplanting other high-risk groups. For example, a 70-year old recipient faces a 30 percent higher risk of graft loss at one year than a 30-year old recipient. However, the PSRs account for this, enabling a center to offer transplantation to the 70-year old without fear of adverse regulatory actions provided that its outcomes are on par with other centers transplanting 70-year olds (49). However, this is not the case for ILDKT: if risk does indeed exist with this practice, that risk is not currently captured in the PSRs, potentially increasing a center's risk of flagging and investigation by the Centers for Medicare & Medicaid Studies (CMS).

We hypothesized that ILDKT is associated with an increased risk of graft loss and death compared to compatible live donor kidney transplantation. To quantify this risk in the context of the PSRs, we created a multicenter collaboration linking anti-HLA DSA strength of ILDKT recipients to other data already reported to the Scientific Registry of Transplant Recipients (SRTR). Then, to examine the effect of ILDKT on the risk of CMS flagging, we conducted stochastic simulations of CMS center evaluations based on the risk associated with ILDKT.

METHODS

Study Population and Incompatible Live Donor Kidney Transplantation Definition

We studied adult (≥ 18 years of age), live donor, kidney-only recipients from 22 U.S. transplant centers through December 2011. The population included all ILDKT performed at a given center as well as all compatible live donor kidney transplants performed at that center since the time they began doing ILDKT (in other words, from the date of each center's first ILDKT; the earliest ILDKT was performed in September 1997). Participating transplant centers provided the antibody strength prior to desensitization for ILDKT recipients, categorized as pre-transplant positive Luminex, negative flow crossmatch (PLNF), positive flow, negative cytotoxic crossmatch (PFNC), or positive cytotoxic crossmatch (PCC). In some cases these were actual cell-based crossmatches, in others, they were virtual crossmatches based on semi-quantitative DSA strength on solid phase assays. Patients who had anti-HLA DSA and were also ABO-incompatible ($n=60$, 5.8% of HLA-incompatible cohort) were considered part of the ILDKT population and were categorized based on the strength of the anti-HLA DSA as described above; a sensitivity analysis excluding these patients did not change any of our inferences.

At Johns Hopkins reactions with test beads yielding mean fluorescence intensity (MFI) of ≥ 1000 have been considered Luminex positive. The range of MFI values reported to result in a positive flow crossmatch is quite broad (2,000-20,000), and may be dependent on antibody class and specificity. PCC results have been associated with $\geq 10,000$ MFI on phenotype panels. Significant variation in the results of solid phase assays within and especially between laboratories has been well characterized (59, 60). Each laboratory

established its own MFI benchmarks that equate to the three crossmatch categories reported in this study.

Data Linkage

Data provided by transplant centers were linked to the SRTR for 1) ascertainment of risk factors (other than DSA strength) in a manner consistent with the PSRs, and 2) reliable ascertainment of outcomes (graft loss and death). The SRTR supplements death ascertainment through linkage to the Social Security Death Master File and death and graft loss ascertainment through linkage to data from the Centers for Medicare and Medicaid Services (CMS). The SRTR includes information on all donors, wait-listed transplant candidates, and transplant recipients in the U.S. provided by members of the Organ Procurement and Transplantation Network (OPTN), and has been well-described elsewhere (61). The Health Resources and Services Administration, U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors.

Outcome Definitions

All-cause graft loss was defined by the time between date of transplantation and either date of graft failure (marked by retransplantation, relisting, a return to dialysis, or death) or last date of follow-up with a functioning graft, with administrative censoring at the end of study. Death was defined as the time between date of transplantation and date of death or administrative end of the study.

Missing Data

Because of changes from panel reactive antibody (PRA) to calculated PRA (CPRA) in 2007, we combined all information available about PRA and/or CPRA, and those missing data (6.7%) were assumed to have the mean PRA/CPRA for ILDKT patients at their level of antibody strength. ILDKT patients missing BMI or with BMI outside a realistic range of 14-50 kg/m² (8.0%) were assumed to have a BMI that was the average for their gender and antibody strength. All other variables were missing in <1% of ILDKT patients. Missingness in compatible recipients, serving as a comparison population, was treated using casewise deletion.

Statistical Analysis

Between-group characteristics were compared using chi-square test for categorical variables and ANOVA for continuous variables. Mortality and graft loss were estimated using the Kaplan-Meier method and compared between groups using log-rank testing. Cox models were constructed to mirror (in terms of variable selection and functional form) the SRTR live donor kidney transplant PSR models for graft loss and mortality (49), with inclusion of DSA strength as an additional variable. Hazard ratios were obtained from the models for the time period up to 1-year post-transplant and after 1 year post-transplant, the former for its regulatory implications and the latter for its biologic and clinical relevance. A two-tailed p-value of <0.05 was considered statistically significant. Statistical analysis was performed using Stata 12.0 (StataCorp, College Station, Texas).

Regulatory Flagging Simulation

To quantify the risk of CMS flagging that centers assume when they perform ILDKT, we conducted stochastic simulations of CMS center evaluations as previously described (62) for live donor kidney transplantation, using differing distributions of ILDKT use and risk. In other words, we simulated the likelihood of transplant centers being flagged by CMS for regulatory review, with an assumption (based on our previous survey (55)) that 30% of transplant centers performed no ILDKT, 40% of centers performed ILDKT for 5% of all of their live donor KT, 15% of centers performed ILDKT for 10% of all of their live donor KT, and the remaining 15% of centers performed ILDKT for 20% of all of their live donor KT. We conducted separate simulations using the all-cause graft loss risk estimates for PFNC and PCC based on the data censored at 1 year. Each simulation was run for 1000 iterations. Probability of flagging was calculated based on centers' proportion of ILDKT as a function of their overall live donor kidney transplant volume and centers' "risk quotient" (a number assigned to each center representing center quality, such that a center with average performance had a risk quotient of 1.0 and a center with 50% higher risk had a risk quotient of 1.5). The risk quotient was randomly assigned and then centers were randomly assigned an ILDKT volume as a percent of overall live donor kidney transplant volume (0%, 5%, 10%, or 20%). The effect of ILDKT on flagging risk was evaluated using logistic regression.

RESULTS

Study Population

Across the 22 participating centers, 10,694 live donor kidney transplants were performed. Of these transplants, 1,025 (9.6%) were ILDKT, including 185 PLNF, 536 PFNC, and 304 PCC transplants. ILDKT patients were more likely to be female (39.1% of compatible, 67.6% of PLNF, 68.1% of PFNC, and 64.8% of PCC patients; $P<0.001$), more likely to be covered by public insurance (38.6% of compatible, 42.7% of PLNF, 52.4% of PFNC, and 59.5% of PCC patients; $P<0.001$), and were less frequently dialysis-free at the time of transplantation (32.0% of compatible, 16.2% of PLNF, 13.2% of PFNC, and 8.2% of PCC patients; $P<0.001$) (Table 1). ILDKT patients were more likely to have previously received a transplant (14.7% of compatible, 36.8% of PLNF, 43.7% of PFNC, and 56.6% of PCC patients; $P<0.001$) and less likely to have undergone zero-HLA-mismatch transplantation (9.1% of compatible, 2.2% of PLNF, 1.7% of PFNC, and 1.3% of PCC patients; $P<0.001$). ILDKT patients also had substantially higher median peak PRA/CPRA values (0 [IQR: 0-7] for compatible, 51 [IQR: 18-82] for PLNF, 58 [IQR: 13-93] for PFNC, and 85 [IQR: 50-98] for PCC patients).

Graft Loss

One-year unadjusted all-cause graft loss was 3.9%, 3.8%, 6.9%, and 19.4% for compatible, PLNF, PFNC, and PCC patients, respectively ($P<0.001$). Five-year unadjusted all-cause graft loss was 16.6%, 20.2%, 28.8%, and 39.9% for compatible, PLNF, PFNC, and PCC patients, respectively (Figure 1). Compared to compatible patients, PLNF patients had a similar adjusted risk of all-cause graft loss in the first year post-transplant (aHR=0.91; 95% CI: 0.43-1.94; $P=0.81$); however, PFNC (aHR=1.64; 95% CI: 1.15-2.33; $P=0.007$) and PCC (aHR=5.01; 95% CI: 3.71-6.77; $P<0.001$) patients

had a significantly higher risk of all-cause graft loss in the first year post-transplant (Table 2). After the first year post-transplant, PLNF patients continued to have a similar adjusted risk of all-cause graft loss compared to compatible patients (aHR=1.20; 95% CI: 0.83-1.75; P=0.33); however, PFNC (aHR=1.65; 95% CI: 1.36-1.99; P<0.001) and PCC (aHR=1.80; 95% CI: 1.42-2.29; P<0.001) patients continued to have a significantly higher risk of all-cause graft loss.

Mortality

One-year unadjusted mortality was 2.0%, 1.6%, 3.9%, and 8.9% for compatible, PLNF, PFNC, and PCC patients, respectively (P<0.001). Five-year unadjusted mortality was 9.3%, 9.6%, 12.9%, and 19.1% for compatible, PLNF, PFNC, and PCC patients, respectively (Figure 2). Compared to compatible patients, PLNF patients had a similar risk of death in the first year post-transplant (aHR=0.83; 95% CI: 0.26-2.62; P=0.75); however, PFNC (aHR=2.04; 95% CI: 1.28-3.26; p=0.003) and PCC (aHR=4.59; 95% CI: 2.98-7.07; P<0.001) patients had a significantly higher risk of death (Table 2). After the first year post-transplant, PLNF patients continued to have a similar adjusted risk of death compared to compatible patients (aHR=0.84; 95% CI: 0.48-1.48; P=0.55); however, PFNC (aHR=1.32; 95% CI: 1.02-1.70; P=0.037) and PCC (aHR=1.51; 95% CI: 1.13-2.03; P<0.001) patients continued to have a significantly higher risk of death.

Regulatory Flagging Simulation

In the stochastic simulation, increased ILDKT volume and increased antibody strength were associated with significantly increased odds of flagging for further regulatory

scrutiny. Compared to centers of equal quality that performed no ILDKT, centers that performed 5%, 10%, or 20% PFNC ILDKT had a 1.19-fold (95% CI: 1.15-1.23), 1.33-fold (95% CI: 1.28-1.39), and 1.73-fold (95% CI: 1.66-1.80) higher odds of CMS flagging, and centers which performed 5%, 10%, or 20% PCC ILDKT had a 2.22-fold (95% CI: 2.14-2.32), 4.09-fold (95% CI: 3.91-4.28), and 10.72-fold (10.27-11.18) higher odds of CMS flagging than their equal quality counterparts (Table 3). Different centers in the simulation have different "risk quotients"--a measure of center quality such that centers with a higher risk quotient have higher risk of graft loss within one year. A center with average performance (risk quotient=1) had a 0.5% chance of getting flagged if it performed no ILDKT, but an 8.5% chance of getting flagged if it performed 20% PCC ILDKT. A center with 50% higher risk of adverse outcomes (risk quotient=1.5) had a 4.7% chance of getting flagged if it performed no ILDKT, but a 29.0% chance of getting flagged if it performed 20% PCC ILDKT (Figure 3).

DISCUSSION

In this 22-center United States study of ILDKT, increased anti-HLA DSA strength was associated with worse graft outcomes and higher mortality following live donor kidney transplantation. Within the context of the SRTR models used by CMS, PLNF patients were similar to compatible patients in terms of all-cause graft loss and death. PFNC and PCC were associated with higher graft loss (aHR=1.64; 95% CI: 1.15-2.33; P=0.007 and aHR=5.01; 95% CI: 3.71-6.77; P<0.001, respectively). Our simulation of CMS regulatory flagging suggested that an increasing proportion of a center's ILDKT volume

led to significant increases in the odds of being flagged, even in the absence of poor performance. Centers that performed PFNC ILDKT as 20 percent of their volume were expected to have a 1.73-fold (95% CI: 1.66-1.80) higher odds of being flagged, and those that performed PCC ILDKT as 20 percent of their volume were expected to have a 10.72-fold (95% CI: 10.27-11.18) higher odds of being flagged.

The ILDKT literature has thus far been based on single-center data with insufficient sample sizes to quantify risk in a precise, generalizable manner (15, 34, 56-58, 63-79). In addition, most of these single-center studies have been descriptive, with absent or limited control groups. Our findings of increased risk of graft loss and death with increasing level of antibody strength, are consistent with previous single-center studies (20, 80). Other studies have compared the outcomes of ILDKT to compatible live donor kidney transplantation, albeit with much less power to adjust for multiple confounding factors (35, 56-58). Haririan and colleagues found that 41 patients with a positive flow crossmatch had a 2.6-fold higher risk of graft loss compared to patients matched on gender, race, age, prior kidney transplant, and year of transplantation. The Mayo Clinic group reported a 5-year death-censored graft survival rate of 70.7% in 102 ILDKT patients, compared to 96.7% in compatible recipients matched for age and sex. They also found that PCC patients had a higher incidence of graft loss than PFNC patients at 1 year, although that difference was not statistically significant by 5 years.

Our study found no difference in patient or graft survival outcomes for PLNF patients compared to compatible patients. In a retrospective study of deceased and live donor

kidney transplants, Gibney and colleagues reported that patients with DSA detected by Luminex but negative by cytotoxic crossmatch had worse graft survival but similar patient survival compared to compatible kidney transplant recipients (81). However, the authors did not report flow cytometric crossmatch results on these patients, so it remains unclear if the difference in graft survival in their study was because some of the patients were actually PFNC. Consistent with our findings, Loupy and colleagues showed equivalent graft and patient outcomes among recipients of deceased donor kidneys with pre-transplant DSA detected by solid-phase assay compared to those without DSA (82). However, the wide confidence intervals for PLNF estimates suggest that the risk associated with this strength of anti-HLA antibody remains incompletely characterized.

The strengths of this study include its multicenter design (22 centers), large sample size (n=10,694), and control group (compatible kidney transplant recipients at the same center, adjusted for SRTR variables, with the statistical power to accommodate all of these variables in a regression model). Participating centers represent more than one-fifth of the 2011 U.S. live donor kidney transplant volume, permitting quantification of the risk associated with increasing antibody strength without the limitations associated with existing single-center reports.

Limitations include heterogeneity across the 22 centers with respect to HLA antibody testing and interpretations of these assays, especially when comparing DSA strength (83). Significant variation exists in the management of interfering agents such as auto-antibody and therapeutic agents in solid phase immunoassays and their subsequent interpretation

(84). Even under tightly controlled circumstances, there is still significant variability in the assessment of antibody strength (85). Centers have their own thresholds of mean fluorescence intensity and mean channel shifts that constitute a positive crossmatch. For cell-based assays, there is significant center-level variation in the conduct and materials of the crossmatch, and also in the threshold for positivity (86). However, our study design accounted for this in two ways. First, we asked each center to classify each patient as PLNF, PFNC, and PCC in order to find a common classification system and allow for the development of the largest cohort of ILDKT patients to date. Second, we only included patients who underwent desensitization, so as to only study those that the centers felt had sufficiently strong DSA to warrant the risks and costs of desensitization. Desensitization strategies, treatment of antibody-mediated rejection, and management of persistent post-operative DSA vary across centers. This study was not designed to assess these differences, nor to determine the best management of these clinical challenges; however, it does provide a real-world snapshot of the aggregate outcomes that result from these various practices.

This multicenter study of ILDKT quantifies the risk for both patients and transplant centers associated with desensitizing and transplanting patients with pre-existing DSA. Even after adjusting for all of the variables in the PSRs, there is a 1.64 and 5.01-fold increase in the risk of graft loss and a 2.04 and 4.59-fold increase in the risk of death for PFNC and PCC patients, respectively, in the first year post-transplant. PFNC and PCC patients continued to have elevated risk of graft loss and death after the first year post-transplant compared to compatible patients. For centers that perform 20% PFNC and

20% PCC as percentages of their overall live donor kidney transplant volume, there is a 1.73 and 10.72-fold increase in the odds of flagging by CMS compared to centers of equal quality that do not perform ILDKT. These findings warn of significant regulatory risk associated with ILDKT. Confirmation of our previous single-center demonstration of ILDKT survival benefit (15) on a multi-center level is necessary to better inform whether the increased regulatory scrutiny is justified or biased against beneficial treatment based on best evidence. The outcomes also serve as a benchmark for the assessment of the efficacy of new innovations and best practices for the relatively young field of HLA-incompatible kidney transplantation.

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The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

The data reported here have been supplied by the Minneapolis Medical Research Foundation (MMRF) as the contractor for the Scientific Registry of Transplant Recipients (SRTR). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

Table 1. Characteristics of Transplant Recipients, by Anti-HLA Antibody Strength

	Compatible (n=9,669)	Positive Luminex, Negative Flow Crossmatch (n=185)	Positive Flow, Negative Cytotoxic Crossmatch Positive (n=536)	Positive Cytotoxic Crossmatch (n=304)	P-Value
Mean Age at Transplant (SD)	47.8 (13.8)	45.4 (12.7)	45.5 (12.6)	43.8 (13.2)	0.17
Female Sex	39.1%	67.6%	68.1%	64.8%	0.006
Recipient Race					0.001
White	68.3%	64.9%	67.7%	73.4%	
Black	15.2%	17.3%	18.7%	11.8%	
Hispanic/Latino	10.9%	9.2%	9.1%	11.8%	
Asian	4.7%	7.6%	3.0%	2.6%	
Public Insurance	38.6%	42.7%	52.4%	59.5%	0.001
Prior Transplant	14.7%	36.8%	43.7%	56.6%	<0.001
Zero HLA Mismatch	9.1%	2.2%	1.7%	1.3%	0.89
Median Peak PRA/CPRA (IQR)	0 (0-7)	51 (18-82)	57.5 (14-93)	85 (50-98)	<0.001
Mean Recipient BMI (SD)	27.5 (5.8)	26.2 (5.4)	26.6 (6.2)	25.6 (5.5)	0.10
ESRD Diagnosis					0.073
Glomerular Disease	27.7%	36.2%	36.7%	35.2%	
Diabetes	23.5%	22.2%	14.2%	10.2%	
Hypertension	19.2%	7.6%	12.5%	15.8%	
Polycystic Kidney Disease	10.4%	10.3%	9.5%	8.2%	
Vascular Disease	1.4%	1.9%	1.7%	2.0%	
Other	17.8%	22.7%	25.4%	28.6%	
Time on Dialysis Prior to Recent Transplant					<0.001
Preemptive	32.0%	16.2%	13.2%	8.2%	
<2 years	41.8%	28.6%	28.5%	16.8%	
2-6 years	14.4%	22.2%	18.1%	19.1%	
≥6 years	11.9%	33.0%	40.1%	55.9%	
Recipient HCV	3.7%	8.1%	6.7%	5.3%	0.50
Mean Donor Age (SD)	42.0 (11.7)	41.6 (11.3)	40.5 (11.7)	40.5 (11.8)	0.57
Living Related Donor	42.8%	55.1%	45.7%	43.4%	0.04
Donor Race					<0.001
White	70.9%	69.2%	68.3%	76.3%	
Black	13.5%	14.0%	17.5%	8.5%	
Hispanic/Latino	10.6%	8.1%	9.7%	11.2%	
Asian	4.0%	8.1%	2.8%	3.3%	

SD=standard deviation; PRA=panel reactive antibody; CPRA=calculated panel reactive antibody;
ESRD=end stage renal disease; HCV=hepatitis C virus; IQR=interquartile range

Table 2. Adjusted Risk of All-Cause Graft Loss and Mortality in the First Year Post-Transplant and After the First Year By Antibody Strength

Antibody Strength	All-Cause Graft Loss				Mortality			
	aHR ≤1 Year	P-value	aHR >1 Year	P-value	aHR ≤1 Year	P-value	aHR >1 Year	P-value
Compatible	Reference	--	Reference	--	Reference	--	Reference	--
Positive Luminex, Negative Flow Crossmatch	0.91 (0.43-1.94)	0.81	1.20 (0.83-1.75)	0.33	0.83 (0.26-2.62)	0.75	0.84 (0.48-1.48)	0.55
Positive Flow, Negative Cytotoxic Crossmatch	1.64 (1.15-2.33)	0.007	1.65 (1.36-1.99)	<0.001	2.04 (1.28-3.26)	0.003	1.32 (1.02-1.70)	0.037
Positive Cytotoxic Crossmatch	5.01 (3.71-6.77)	<0.001	1.80* (1.42-2.29)	<0.001	4.59 (2.98-7.07)	<0.001	1.51* (1.13-2.03)	<0.001

Risk was adjusted based on the variables in the Scientific Registry of Transplant Recipients 1 year adjustment models for graft loss and mortality.

aHR=adjusted hazard ratio

* Interaction term between antibody strength and time>1 year with P-value<0.001.

Table 3. Effect of Incompatible Live Donor Kidney Transplantation on Risk of Flagging by Centers for Medicare & Medicaid Studies in a Simulation.

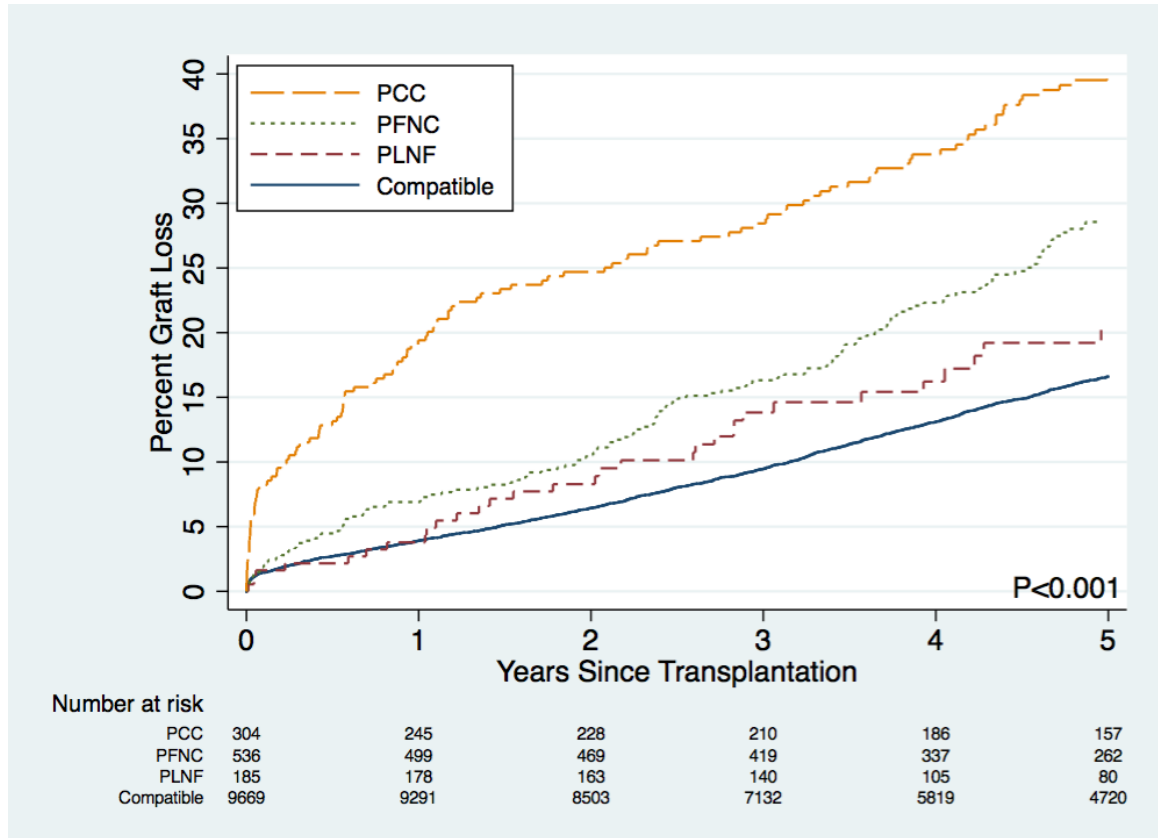
Center Volume of ILDKT	Odds Ratio of Regulatory Flagging	
	Positive Flow, Negative Cytotoxic Crossmatch	Positive Cytotoxic Crossmatch
No ILDKT	Reference	Reference
5 % ILDKT	1.19 (1.15-1.23)	2.22 (2.14-2.32)
10% ILDKT	1.33 (1.28-1.39)	4.09 (3.91-4.28)
20% ILDKT	1.73 (1.66-1.80)	10.72 (10.27-11.18)

This simulation, run 1000 times, estimates the likelihood of transplant centers being flagged by CMS for regulatory review, with an assumption that 30% of transplant centers performed no ILDKT, 40% of centers performed ILDKT for 5% of all of their live donor KT, 15% of centers performed ILDKT for 10% of all of their live donor KT, and the remaining 15% of centers performed ILDKT for 20% of all of their live donor KT. We conducted separate simulations using the all-cause graft loss risk estimates for PFNC and PCC. Probability of flagging was calculated based on centers' proportion of ILDKT as a function of their overall live donor kidney transplant volume and centers' "risk quotient" (a number assigned to each center representing center quality, such that a center with average performance had a risk quotient of 1.0 and a center with 50% higher risk had a risk quotient of 1.5). The risk quotient was randomly assigned and then centers were randomly assigned an ILDKT volume as a percent of overall live donor kidney transplant volume (0%, 5%, 10%, or 15%). The effect of ILDKT on flagging risk was evaluated using logistic regression.

Compared to centers which performed no ILDKT, centers of equal quality which performed 5%, 10%, or 20% PFNC ILDKT had 1.19-fold, 1.33-fold, and 1.73-fold higher odds of CMS flagging, respectively, in a logistic regression model. Centers which performed 5%, 10%, or 20% PCC ILDKT had 2.22-fold, 4.09-fold, and 10.72-fold higher odds of CMS flagging, respectively. All p values are < 0.001.

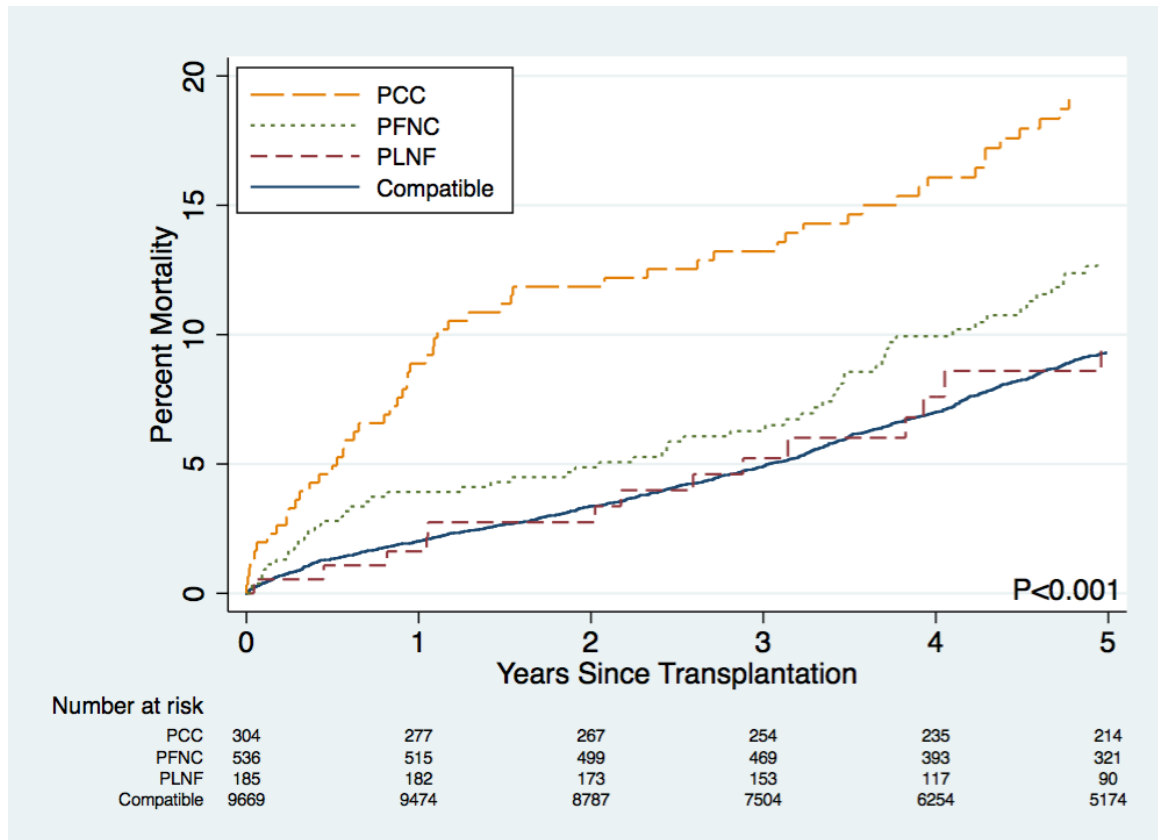
ILDKT=incompatible live donor kidney transplantation; PFNC=positive flow, negative cytotoxic crossmatch; PCC=positive cytotoxic crossmatch

Figure 1. All-Cause Graft Loss, by Antibody Strength



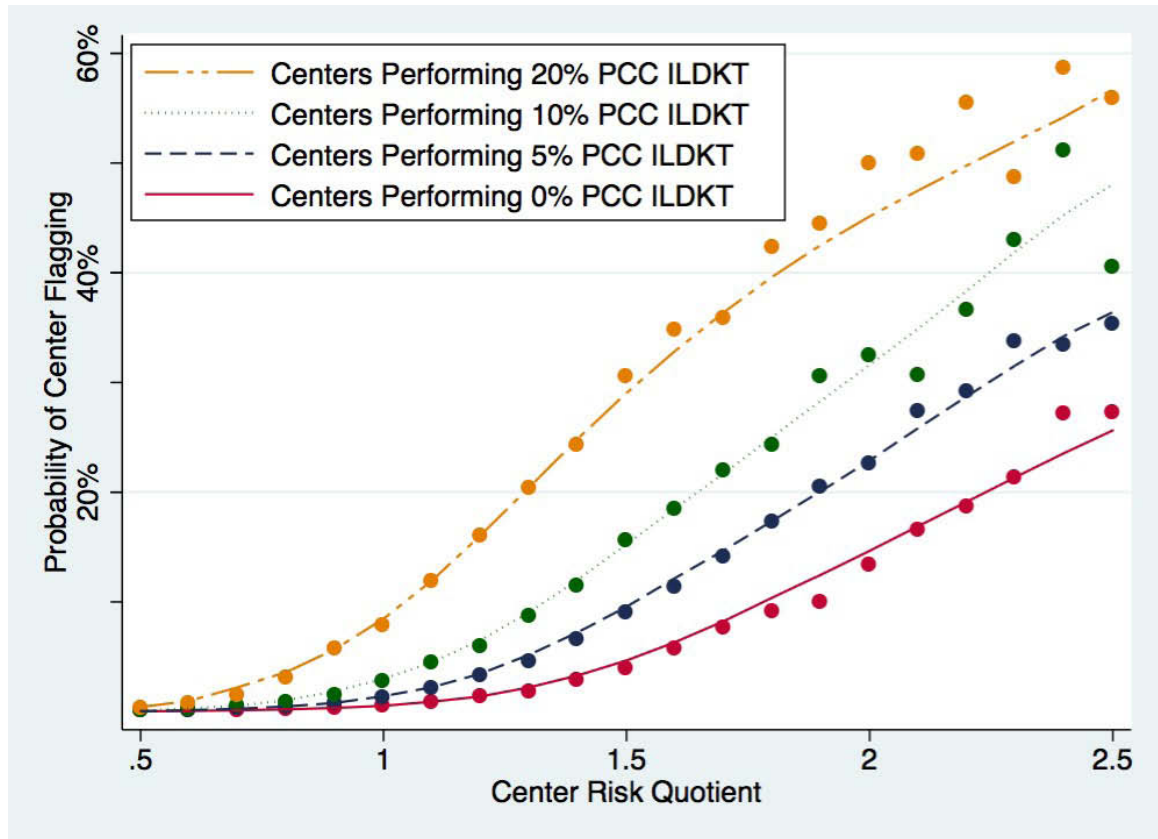
PCC=positive cytotoxic crossmatch; PFNC=positive flow, negative cytotoxic crossmatch; PLNF=positive Luminex, negative flow crossmatch

Figure 2. Post-Transplant Mortality, by Antibody Strength



PCC=positive cytotoxic crossmatch; PFNC=positive flow, negative cytotoxic crossmatch; PLNF=positive Luminex, negative flow crossmatch

Figure 3. The Effect of Incompatible Live Donor Kidney Transplantation on Risk of Flagging by Centers for Medicare & Medicaid Studies in a Simulation.



Different centers in the simulation have different "risk quotients" – a measure of center quality such that centers with a higher risk quotient have higher risk of graft loss within one year. A center with average performance (risk quotient=1) had a 0.5% chance of getting flagged if it performed no ILDKT, but an 8.5% chance of getting flagged if it performed 20% PCC ILDKT. A center with 50% higher risk of adverse outcomes (risk quotient=1.5) had a 4.7% chance of getting flagged if it performed no ILDKT, but a 29.0% chance of getting flagged if it performed 20% PCC ILDKT.

CHAPTER 3 - Quantifying Renal Allograft Loss following Early Antibody-Mediated Rejection

Babak J. Orandi MD PhD MSc¹, Eric H. K. Chow MS¹, Annie Hsu BS¹, Natasha Gupta BS¹, Kyle J. Van Arendonk MD PhD¹, Jacqueline M. Garonzik-Wang MD PhD¹, John R. Montgomery BS¹, Corey Wickliffe BA¹, Bonnie E. Lonze MD PhD¹, Serena M. Bagnasco MD², Nada Alachkar MD³, Edward S. Kraus MD³, Robert A. Montgomery MD DPhil¹, Dorry L. Segev MD PhD¹

Affiliations: Johns Hopkins Medical Institutions, Departments of Surgery¹, Pathology², and Medicine³, Baltimore, MD

ABSTRACT:

While antibody-mediated rejection (AMR) has been implicated in reducing graft survival, the magnitude of this effect is unclear, and it remains unknown if this effect is homogeneous across donor types (deceased/live), antibody types (HLA/ABO), and presentation (clinical/subclinical). We compared 219 patients with biopsy-proven AMR to controls matched on HLA-compatibility, ABO-compatibility, donor type, prior transplant, PRA, age, and transplant year. Graft loss in AMR patients at 1 and 5 years was 14.2% and 28.9%, versus 2.0% and 8.0% in matched controls ($P<0.001$), a finding confirmed by propensity score-matching. AMR was independently associated with 4.61-fold higher graft loss (95% CI: 3.19-6.68, $p<0.001$) compared to matched controls, although this effect was heterogeneous when stratified by HLA-compatible deceased donor (HR=4.73;95%CI:1.57-14.26; $P=0.006$), HLA-incompatible deceased donor (HR=2.39;95%CI:1.10-5.19; $P=0.028$), HLA-compatible live donor (no AMR patients had graft loss), ABO-incompatible live donor (HR=6.13;95%CI:0.55-67.70; $P=0.14$), and HLA-incompatible live donor (HR=6.29;95%CI:3.81-10.39; $P<0.001$) transplant. A subclinical AMR presentation was independently associated with 2.35-fold higher graft loss (95%CI:1.30-4.25; $P=0.005$) compared to matched non-AMR controls. AMR, even without a clinical presentation, substantially increases graft loss, particularly in deceased donor and HLA-incompatible live donor recipients. Aggressive prevention, surveillance, and treatment strategies are warranted in these populations.

INTRODUCTION:

Over 30% of the kidney transplant waitlist is comprised of sensitized patients (PRA \geq 20%) (87). In an effort to transplant these vulnerable patients, advances in desensitization protocols and kidney paired donation have promoted the widespread adoption of incompatible kidney transplantation at centers across the United States (12, 14, 15, 88). While antibody-mediated rejection (AMR) occurs in approximately 5% of compatible kidney transplants, the incidence seems to be significantly higher in incompatible kidney transplant recipients, as high as 40% in some reports (80, 89, 90). As incompatible kidney transplantation and re-transplantation become more common (91), the incidence of antibody-mediated rejection (AMR) can only be expected to increase.

AMR has long been suspected to threaten graft survival. However, the magnitude of this effect is unclear; estimates of the risk of graft loss range from 1.53 (95% CI: 1.21-1.91) to 4.58 (95% CI: 1.75-12.00) (92, 93). Furthermore, it remains unknown, both epidemiologically and mechanistically, if AMR differentially affects graft outcomes depending upon donor compatibility and donor type (deceased versus live donor). Indeed, the 2013 Banff Conference on Allograft Pathology reported that, for the first time, there will be a Banff Working Group formed to evaluate possible differences between non-sensitized patients who develop AMR and patients requiring desensitization who develop AMR (39). Finally, studies to date have suggested that patients with a subclinical presentation of AMR have poorer graft function and worse pathological findings on subsequent allograft biopsies (46, 82), though an association between subclinical AMR and increased graft loss has yet to be explored.

The objective of this study was to understand the risk of allograft loss associated with AMR using a well-defined set of clinicopathologic criteria. We also sought to quantify the risk of graft loss following AMR by the type of transplant (e.g., HLA-compatible deceased donor, HLA-incompatible deceased donor, HLA-compatible live donor, ABO-incompatible live donor, HLA-incompatible live donor) performed as well as its presentation (subclinical or clinical).

METHODS:

Study Population

We studied all adult (≥ 18 years of age), kidney-only transplants performed at the Johns Hopkins Hospital from January 2000 through December 2012 (n=2,317). HLA-incompatible live donor recipients were defined as those who had anti-HLA DSA at a strength requiring perioperative desensitization therapy. Deceased donor HLA-incompatible recipients were those who had the presence of anti-HLA DSA identified at the time of transplant, but with a negative cytotoxic crossmatch. Patients who were ABO-incompatible with their donor and also had anti-HLA DSA were studied as members of the HLA-incompatible live donor group. This study was approved by the Johns Hopkins Medical Institutions Institutional Review Board.

Peak PRA/CPRA

Because of OPTN policy changes from PRA to CPRA in 2007, we combined all information available about PRA and/or CPRA, and those missing data (2.8%) were imputed to have the mean PRA/CPRA for patients of their same transplant type.

Performance of Biopsies:

Ultrasound-guided needle allograft biopsies were performed as previously described (94). Occasionally, biopsies were performed during an open re-operation under direct visualization. C4d staining was performed on frozen tissue using indirect immunofluorescence with anti-human C4d antibody (Quidel, San Diego, CA) at 1:40 dilution followed by secondary antibody (fluorescein isothiocyanate-conjugated goat anti-mouse IgG; Jackson ImmunoResearch Laboratories, West Grove, PA) or on paraffin-embedded tissue sections using a rabbit polyclonal anti-human antibody (American, Pfungstadt, Germany) at a 1:50 dilution, in concert with a biotin-free polymer detection system (Leica, Wetzlar, Germany).

Diagnosis of AMR:

The diagnosis of AMR occurring in the first year post-transplant was based on the 2013 international Banff Classification Criteria (39) and defined as the presence of circulating DSA and either: 1) peritubular capillary staining of C4d and at least one of the following: ptc score>0, g score>0, acute thrombotic microangiopathy in the absence of any other cause, or other features consistent with AMR (endothelial injury, fibrin thrombi, microinfarctions, interstitial hemorrhage), or 2) absence of capillary staining of C4d and the presence of ptc>0 and g>0 or ptc>0 or g>0 and acute thrombotic microangiopathy, in

the absence of any other cause of thrombotic microangiopathy. Index episodes of AMR were also noted to be clinical or subclinical. Clinical episodes of AMR were defined as those that had evidence of graft dysfunction, manifested as oliguria/anuria, an increase in serum creatinine by $\geq 20\%$ from baseline, treatment of cell-mediated rejection and/or thrombotic microangiopathy within the two prior weeks, or new onset proteinuria.

Matched Controls Selection

Patients who developed AMR were matched to those who did not develop AMR using iterative expanding radius matching as previously described (15, 95, 96). AMR patients were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak PRA/CPRA, age at transplant, and year of transplant.

Outcome Ascertainment

Death-censored graft survival (DCGS) was defined as the time between date of transplant and either date of graft failure (marked by retransplantation, relisting, or a return to dialysis) or last date of follow-up with a functioning graft, censoring for death and administrative end of study. Death and graft failure ascertainment were augmented through linkage with the Social Security Death Master File and the Centers for Medicare and Medicaid Services by the Scientific Registry of Transplant Recipients (SRTR). The SRTR includes information on all donors, wait-listed transplant candidates, and transplant recipients in the U.S. provided by members of the Organ Procurement and Transplantation Network (OPTN), and has been well-described elsewhere (97). The

Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors.

Statistical Analysis

Between-group characteristics were compared using chi-square test for categorical variables, ANOVA for normally distributed continuous variables, and the Wilcoxon rank sum test for non-normally distributed continuous variables. Death-censored graft survival was estimated using the Kaplan-Meier method and compared between groups using log-rank testing and Cox models. Proportional hazards assumptions were confirmed by visual inspection of complimentary log-log plots. Separate Cox models were performed by the type of transplant (HLA-compatible/incompatible live donor transplant, HLA-compatible/incompatible deceased donor transplant, ABO-incompatible live donor transplant). Analyses were performed using Stata 12.1/SE (Stata Corp, College Station, TX). A two-tailed p-value less than 0.05 was considered statistically significant.

Propensity Score Analysis

As a sensitivity analysis, AMR patients were compared with non-AMR controls matched on propensity for developing AMR. Propensity scores were generated using R (R Foundation for Statistical Computing, Vienna, Austria) with random forest methods, and 1:1 matches were assigned using nearest neighbor matching. Subsequent analyses were performed with Stata. Graft survival was estimated using the Kaplan-Meier method and compared between AMR patients and propensity score-matched non-AMR controls using

log-rank testing and Cox models. There was no difference in the inferences of the sensitivity analysis and those of the main analysis described above.

RESULTS:

Study Population

Of 2,316 patients who underwent kidney-only transplantation during the study period, 219 developed biopsy-proven AMR (9.5%). AMR patients were more frequently female (58.9% versus 42.9%), had a higher median peak PRA/CPRA (80 versus 2), spent more time on dialysis prior to the transplant (56.2% on dialysis \geq 6 years versus 18.1%), and were more likely to have undergone a previous transplant (55.2% versus 16.7%) (Table 1). AMR patients were more likely have been the recipients of HLA-incompatible live donor transplants (63.0% versus 7.6%), ABO-incompatible live donor transplants (16.4% versus 4.2%), and HLA-incompatible deceased donor transplants (19.6% versus 2.6%).

AMR Incidence

The incidence of AMR was 1.9% in HLA-compatible deceased donor transplant recipients, 44.3% in HLA-incompatible deceased donor transplant recipients, 0.7% in HLA-compatible live donor transplant recipients, 14.8% in ABO-incompatible live donor transplant recipients, and 45.1% in HLA-incompatible live donor transplant recipients.

Death-Censored Graft Survival

Death-censored graft survival in AMR patients was 85.8% at 1 year and 71.1% at 5 years, 98.0% and 92.0% for matched non-AMR controls ($P < 0.001$) (Figure 1, Table 2).

Graft Loss by Transplant Type

Recipients who developed AMR had a 4.61-fold higher risk of graft loss (95% CI: 3.19-6.68; $P<0.001$) (Table 3A) when compared to matched controls. This risk varied by donor type and compatibility, with 4.73-fold higher risk (95% CI: 1.57-14.26; $P=0.006$) in HLA-compatible deceased donor recipients, 2.39-fold higher risk (95% CI: 1.10-5.19; $P=0.028$) in HLA-incompatible deceased donor recipients, 6.13-fold higher risk (95% CI: 0.55-67.70; $P=0.14$) in ABO-incompatible live donor recipients, and 6.29-fold higher risk (95% CI: 3.81-10.39; $P<0.001$) in HLA-incompatible live donor recipients. No HLA-compatible live donor recipients with AMR experienced graft loss.

Subclinical Presentations of Antibody-Mediated Rejection

Of patients who developed AMR, 39.3% had a subclinical presentation of AMR at the time of their AMR-defining biopsy. By transplant type, 25.0% of HLA-compatible deceased donor recipients, 32.6% of HLA-incompatible deceased donor recipients, 50.0% of HLA-compatible live donor recipients, 50.0% of ABO-incompatible live donor recipients, and 42.0% of HLA-incompatible live donor recipients who had AMR had subclinical presentations of AMR (Table 3B).

Graft Outcomes in Subclinical AMR

Death-censored graft survival in subclinical AMR patients was 95.1% at 1 year and 75.5% at 5 years, versus 96.3% and 89.2% for controls matched to the subclinical patients ($P=0.004$) (Figure 2), and 79.8% at 1 year and 68.0% at 5 years for patients with

a clinical presentation of AMR (Figure 3). The risk of graft loss for patients developing subclinical AMR was 2.35-fold (95% CI: 1.30-4.25; P=0.005) higher than for matched controls without AMR. The risk of graft loss for patients with a clinical presentation of AMR was 1.66-fold (95% CI: 0.96-2.85; P=0.067) higher than for patients with a subclinical presentation of AMR.

DISCUSSION:

In this single-center study of 219-patients who developed biopsy-proven AMR consistent with 2013 Banff diagnostic criteria, we found that AMR was associated with an overall 4.61-fold (95% CI: 3.19-6.68; P<0.001) increase in the risk of graft loss. Specifically, the risk of graft loss for AMR patients was 4.73-fold (95% CI: 1.57-14.26; P=0.006) higher in HLA-compatible deceased donor recipients, 2.39-fold (95% CI: 1.10-5.19; P=0.028) higher in HLA-incompatible deceased donor recipients, 6.13-fold (95% CI: 0.55-67.70; P=0.14) higher in ABO-incompatible live donor recipients, and 6.29-fold (95% CI: 3.81-10.39; P<0.001) higher in HLA-incompatible live donor recipients. No HLA-compatible live donor recipients with AMR experienced graft loss. The risk of graft loss for patients with a subclinical presentation of AMR was 2.35-fold (95% CI: 1.30-4.25; P=0.005) higher than for matched controls without AMR. In turn, the risk of graft loss for patients with a clinical presentation of AMR was 1.66-fold (95% CI: 0.96-2.85; P=0.067) higher than for patients with a subclinical presentation of AMR.

The rare occurrence of AMR in HLA-compatible recipients (0.7% live donor and 1.9% deceased donor) in our study is consistent with other single-center studies (98, 99). For example, Everly et al. reported a 1.8% and 1.5% incidence of AMR in HLA-compatible live and deceased donors. The incidences of AMR among deceased donor recipients (44.3% HLA-incompatible and 1.9% HLA-compatible) were consistent with incidences of 43-54% and 5% reported in the literature (44, 100). The high incidence of AMR in HLA-incompatible live donor recipients (46.5%) is also consistent with the 41-53% incidence reported by others (35, 57, 70). AMR occurred in 14.8% of our ABO-incompatible live donor recipients, compared to 40% reported by Fidler and colleagues (101), although this is likely related to the fact that we defined the ABO-incompatible cohort to be purely ABO-incompatible, i.e. ABO-incompatible transplants with anti-HLA DSA were categorized as HLA-incompatible live donor transplants. When all ABO-incompatible transplants were considered, irrespective of anti-HLA DSA, our AMR incidence in ABO-incompatible recipients was a more similar 28.8%. Toki, et al., reported a 33% incidence of AMR in the first 3 months post-transplant in their ABO-incompatible live donor recipients (102).

Our findings of increased graft loss following AMR consolidate evidence of this causal pathway from several other studies. Loupy and colleagues reported that glomerular and peritubular inflammation, transplant glomerulopathy, and post-transplant C1q-binding DSA (pathologic and immunologic factors of AMR) on 1-year protocol biopsies were independently associated with graft loss (47). Lefaucheur et al. reported a 4.1-fold (95% CI: 2.2-7.7) higher risk of graft loss in HLA-incompatible deceased donor recipients who

developed AMR, consistent with but higher than our finding of a 2.39-fold (95% CI: 1.10-5.19) higher risk (103). Couzi and colleagues reported a trend toward worse graft survival in this population as well (100). Dunn and colleagues also demonstrated a 2.9-fold increase in the risk of graft loss for live and deceased donor recipients with pre-transplant DSA (35). In a study of HLA-incompatible live donor recipients, Gloor and colleagues reported a nearly 3-fold increase in graft loss in AMR patients,, compared to our finding of a 6.3-fold increase in the risk of graft loss (57). Among ABO-incompatible live donor recipients, however, Fidler, et al., did not find increased graft loss following AMR, nor did they find an increased incidence of chronic allograft nephropathy on 1-year protocol biopsies (101). While Toki, et al., reported significantly worse graft survival in ABO-incompatible live donor AMR patients, all of the patients who experienced graft loss also had pre-transplant anti-HLA DSA (102), so these inferences are not generalizable to pure ABO-incompatibility. We did not detect a statistically significant difference in the risk of graft loss for ABO-incompatible patients who develop AMR (HR: 6.13; 95% CI: 0.55-67.70); however, the wide confidence interval for this estimate suggests that the risk associated with AMR in ABO-incompatible recipients remains incompletely characterized.

Our finding of subclinical AMR associated with worse graft survival is novel, and consistent with previous findings of worse graft function and histologic findings in patients who previously had subclinical AMR. Our group has previously reported that subclinical AMR is associated with the development of chronic allograft nephropathy (46). Loupy and colleagues, in a study of HLA-incompatible deceased donor recipients,

reported a number of adverse associations with subclinical AMR, including lower glomerular filtration rate, higher serum creatinine, and more peritubular capillaritis, C4d deposition, and transplant glomerulopathy on biopsy, and worse all-cause graft survival at 4 years post-transplant (82).

Strengths of this investigation include the timely, rigorous criteria for AMR consistent with the Banff 2013 criteria and the fact that this is the largest known cohort of patients with AMR (39). Limitations of this study include the possibility of misclassification of AMR, particularly in the event of subclinical AMR, which is less likely to prompt a for-cause biopsy. However, such cases of AMR (that do not result in clinical renal dysfunction) are likely to be the least harmful to graft survival, so this misclassification, if present, would likely bias towards the null, rendering our estimates of the risk of graft loss conservative. Furthermore, we performed protocol biopsies at 1, 3, 6, and 12 months following ABO- and HLA-incompatible live donor transplants, but not routinely after deceased donor transplants or compatible live donor transplants, introducing possible ascertainment bias. Finally, because this study includes patients from a single institution, there may be some limits to external validity (i.e. generalizability to the general transplant population).

CONCLUSION:

The development of AMR is associated with a significantly higher incidence of allograft loss, although the effects of AMR differ by transplant type. HLA-compatible live donor recipients who develop AMR do not have a higher risk of graft loss compared to their

counterparts who do not develop AMR, and the risk for recipients of ABO-incompatible transplants remains inconclusive. However, deceased donor (both HLA-compatible and HLA-incompatible) and HLA-incompatible live donor recipients do have significantly higher risks of graft loss following AMR. Subclinical AMR also poses a threat to graft survival. These data highlight the need for strategies to prevent and aggressively treat AMR, as well as performance of protocol biopsies in high-risk patients.

AUTHOR CONTRIBUTIONS:

Study concept and design: BJO, JRM, RAM, DLS

Acquisition of data: BJO, EHKC, AH, NG, JRM, BEL, NA, SMB, RAM

Analysis and interpretation of data: BJO, EHKC, KJVA, AH, NG, JRM, JMGW, BEL, NA, SMB, RAM, DLS

Critical revision of the manuscript for important intellectual content: BJO, EHKC, KJVA, AH, NG, JRM, JMGW, BEL, NA, SMB, RAM, DLS

Statistical analysis: BJO, EHKC, DLS

Obtained funding: NA

Administrative, technical, or material support: N/A

Study supervision: BJO, RAM, DLS

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Online-Only Material (if any): NA

Table 1. Patient Characteristics of the Overall Cohort, the Antibody-Mediated Rejection Group, and the Matched Controls

	Overall (n=2,316)	Non-AMR Patients (n=2,097)	AMR Patients (n=219)	Matched Controls‡ (n=657)	P- Value*
TRANSPLANT TYPE					
Deceased Donor	48.7%	50.7%	28.8%	25.7%	0.4
HLA-Incompatible	4.2%	2.6%	19.6%	16.4%	0.3
Live Donor	51.3%	49.3%	71.2%	74.3%	0.4
ABO-Incompatible	5.4%	4.2%	16.4%	12.6%	0.1
HLA-Incompatible	12.8%	7.6%	63.0%	62.7%	0.9
PATIENT CHARACTERISTICS					
Age at Transplant (SD)	50.3 (14.2)	50.8 (14.2)	46.0 (13.4)	46.0 (11.9)	0.8
Female	44.4%	42.9%	58.9%	59.7%	0.8
African-American	33.8%	34.8%	24.7%	25.4%	0.8
Public Insurance	47.1%	45.2%	65.7%	52.2%	0.002
BMI (SD)	27.3 (6.0)	27.5 (6.0)	25.9 (6.1)	26.4 (6.0)	0.3
Cause of ESRD					
Glomerular Diseases	25.3%	23.9%	38.4%	34.2%	0.3
Diabetes Mellitus	17.8%	18.6%	10.3%	11.0%	0.6
Hypertensive Nephrosclerosis	29.9%	31.1%	21.0%	17.2%	0.7
Polycystic Kidney Disease	9.8%	9.8%	9.0%	13.1%	0.2
Renovascular & Other Vascular Diseases	0.9%	0.7%	0.5%	0.9%	0.02
Other or Missing (includes Tubular and Congenital)	16.3%	15.8%	23.3%	21.3%	0.9
Peak PRA/CPRA (IQR)	4 (0-100)	2 (0-33)	80 (23-100)	92 (70-100)	0.9
Previous Transplant	20.4%	16.7%	55.2%	55.1%	1.0
Zero HLA-mismatch	7.2%	7.9%	4.1%	0.9%	0.02
Time on Dialysis Prior to Recent Transplant					0.4
Preemptive	16.7%	17.9%	5.9%	9.4%	
≤2years	30.2%	31.9%	14.2%	15.4%	
2-6 years	31.3%	32.1%	23.7%	23.6%	
≥6 years	21.7%	18.1%	56.2%	51.6%	
Donor Age (SD)	40.8 (14.6)	40.7 (14.8)	42.2 (12.9)	40.3 (14.0)	0.08

*Refers to the statistical test that tested the difference between AMR patients and matched controls.

‡ Patients who developed AMR were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak panel-reactive antibody/calculated panel reactive antibody, age at transplant, and year of transplant to patients who did not develop AMR.

AMR=antibody-mediated rejection, SD=standard deviation, BMI=body mass index, ESRD=end stage renal disease, IQR=interquartile range, PRA/CPRA=panel reactive antibody/calculated panel reactive antibody

Table 2. Estimates of Death-Censored Graft Survival

	Death-Censored Graft Survival		P-Value
	1 Year (95% Confidence Interval)	5 Year (95% Confidence Interval)	
AMR	85.8% (80.3-89.8%)	71.1% (63.3-77.6%)	
Non-AMR Matched Controls*	98.0% (96.5-98.8%)	92.0% (89.0-94.2%)	<0.001 [†]
Non-AMR Propensity Score Matched Patients	98.5% (95.5-99.5%)	88.1% (79.1-93.4%)	<0.001 [§]
AMR--Clinical Presentation [‡]	79.8% (71.7-85.8%)	68.0% (58.0-76.1%)	0.067 [‡]
AMR--Subclinical Presentation	95.1% (87.4-98.1%)	75.5% (61.2-85.2%)	
Non-AMR Matched Controls ⁺	96.3% (93.1-98.1%)	89.2% (83.2-93.2%)	0.004

* Patients who developed AMR were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak panel-reactive antibody/calculated panel reactive antibody, age at transplant, and year of transplant to patients who did not develop AMR.

† Refers to the statistical test between AMR patients and non-AMR matched controls.

§ Refers to the statistical test between AMR patients and non-AMR propensity score matched controls.

‡ Clinical episodes of AMR were distinguished from subclinical episodes of AMR by the presence of evidence of graft dysfunction, manifested as oliguria/anuria, an increase in serum creatinine by $\geq 20\%$ from baseline, treatment of cell-mediated rejection and/or thrombotic microangiopathy within the two prior weeks, or new onset proteinuria.

‡ Refers to the statistical test between AMR patients with a clinical presentation and AMR patients with a subclinical presentation.

+ Patients who developed subclinical AMR were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak panel-reactive antibody/calculated panel reactive antibody, age at transplant, and year of transplant to patients who did not develop AMR.

AMR=antibody-mediated rejection

Table 3A. Antibody-Mediated Rejection Risk of Graft Loss, by Transplant Type

Transplant Type	Hazard Ratio‡	P-value
Deceased Donor: HLA-Compatible	4.73 (1.57-14.26)	0.006
Deceased Donor: HLA-Incompatible	2.39 (1.10-5.19)	0.028
Live Donor: HLA-Compatible	NA§	--
Live donor: ABO-Incompatible ⁺	6.13 (0.55-67.70)	0.1
Live Donor: HLA-Incompatible	6.29 (3.81-10.39)	0.001
Overall	4.61 (3.19-6.68)	<0.001

Table 3B. Antibody-Mediated Rejection Incidence and Presentation

Transplant Type	Incidence of AMR		
	Overall (n=219)	Clinical Presentation* (n=133)	Subclinical Presentation (n=86)
Deceased Donor: HLA-Compatible	1.9%	1.5%	0.5%
Deceased Donor: HLA-Incompatible	44.3%	29.9%	14.4%
Live Donor: HLA-Compatible	0.7%	0.4%	0.4%
Live donor: ABO-Incompatible ⁺	14.8%	7.4%	7.4%
Live Donor: HLA-Incompatible	46.5%	26.9%	19.5%
Overall	9.4%	5.7%	3.7%

The diagnosis of antibody-mediated rejection (AMR) occurring in the first year post-transplantation was based on the 2013 international Banff Classification Criteria (39) and defined as the presence of circulating donor-specific antibody and either: 1) peritubular capillary staining of C4d and at least one of the following: ptc score>0, g score>0, acute thrombotic microangiopathy in the absence of any other cause, or other features consistent with AMR (endothelial injury, fibrin thrombi, microinfarctions, interstitial hemorrhage), or 2) absence of capillary staining of C4d and the presence of ptc>0 and g>0 or ptc>0 or g>0 and acute thrombotic microangiopathy, in the absence of any other cause of thrombotic microangiopathy. Patients who developed AMR were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak panel-reactive antibody/calculated panel reactive antibody, age at transplant, and year of transplant to patients who did not develop AMR.

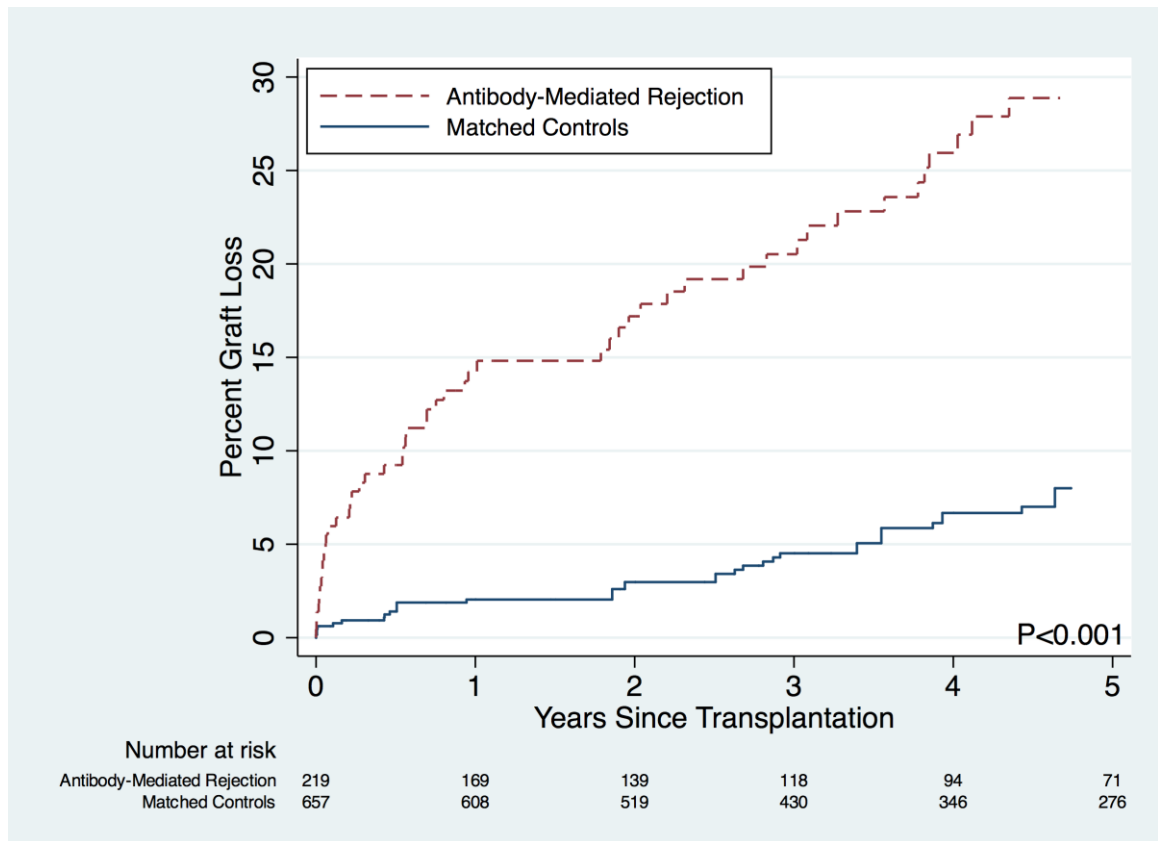
* Clinical episodes of AMR were distinguished from subclinical episodes of AMR by the presence of evidence of graft dysfunction, manifested as oliguria/anuria, an increase in serum creatinine by $\geq 20\%$ from baseline, treatment of cell-mediated rejection and/or thrombotic microangiopathy within the two prior weeks, or new onset proteinuria.

‡ Refers to hazard ratio comparing AMR patients of a transplant type with patients of the same transplant type who did *not* develop AMR.

§ Hazard ratio could not be calculated as no HLA-compatible live donor recipients with AMR had a graft loss during the study period.

⁺ Patients who were ABO-incompatible with their donor and also had anti-HLA donor-specific antibody were considered to be members of the HLA-incompatible live donor group (n=76; 13.8% of the HLA-incompatible live donor recipient population).

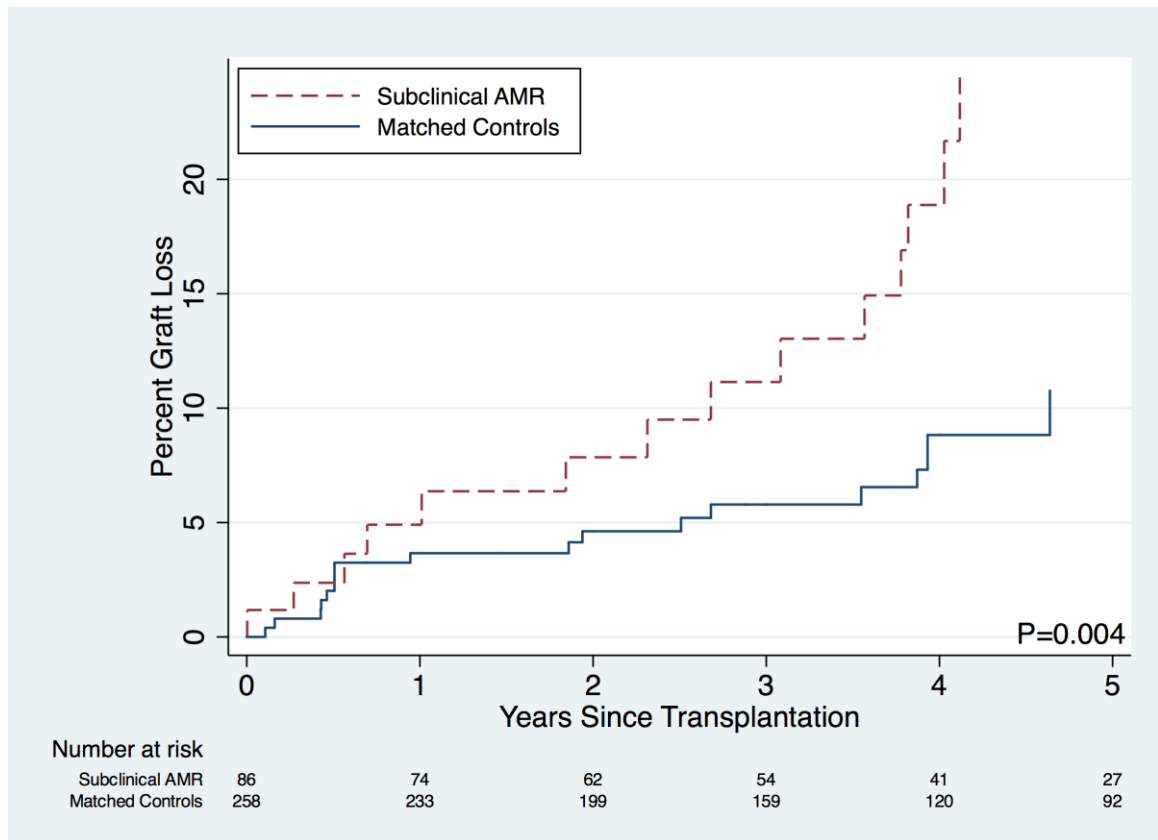
Figure 1. Death-Censored Graft Loss, by Development of Antibody-Mediated Rejection



The diagnosis of antibody-mediated rejection (AMR) occurring in the first year post-transplantation was based on the 2013 international Banff Classification Criteria (39) and defined as the presence of circulating donor-specific antibody and either: 1) peritubular capillary staining of C4d and at least one of the following: ptc score>0, g score>0, acute thrombotic microangiopathy in the absence of any other cause, or other features consistent with AMR (endothelial injury, fibrin thrombi, microinfarctions, interstitial hemorrhage), or 2) absence of capillary staining of C4d and the presence of ptc>0 and g>0 or ptc>0 or g>0 and acute thrombotic microangiopathy, in the absence of any other cause of thrombotic microangiopathy.

Patients who developed AMR were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak panel-reactive antibody/calculated panel reactive antibody, age at transplant, and year of transplant to patients who did not develop AMR.

Figure 2. Death-Censored Graft Loss, by Development of Subclinical Antibody-Mediated Rejection

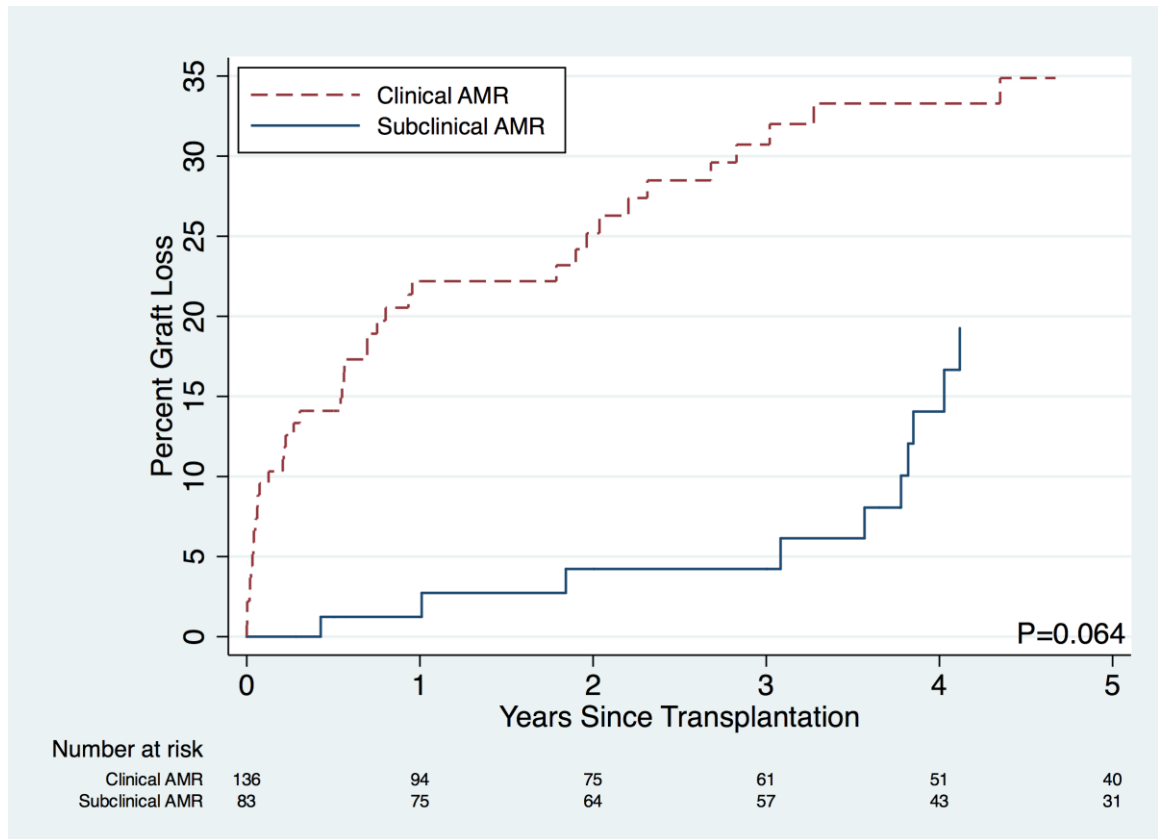


The diagnosis of antibody-mediated rejection (AMR) occurring in the first year post-transplantation was based on the 2013 international Banff Classification Criteria (39) and defined as the presence of circulating donor-specific antibody and either: 1) peritubular capillary staining of C4d and at least one of the following: ptc score>0, g score>0, acute thrombotic microangiopathy in the absence of any other cause, or other features consistent with AMR (endothelial injury, fibrin thrombi, microinfarctions, interstitial hemorrhage), or 2) absence of capillary staining of C4d and the presence of ptc>0 and g>0 or ptc>0 or g>0 and acute thrombotic microangiopathy, in the absence of any other cause of thrombotic microangiopathy.

Patients who developed subclinical AMR were matched in a 1:3 ratio on HLA-compatibility, ABO-compatibility, donor type (deceased versus live), history of previous transplant, peak panel-reactive antibody/calculated panel reactive antibody, age at transplant, and year of transplant to patients who did not develop AMR.

Subclinical episodes of AMR were those that did not have evidence of graft dysfunction, manifested as oliguria/anuria, an increase in serum creatinine by $\geq 20\%$ from baseline, treatment of cell-mediated rejection and/or thrombotic microangiopathy within the two prior weeks, new onset proteinuria, the need for post-transplant splenectomy, or the need for dialysis at the time of the AMR-defining biopsy.

Figure 3. Death-Censored Graft Loss, by Presentation of Antibody-Mediated Rejection



The diagnosis of antibody-mediated rejection (AMR) occurring in the first year post-transplantation was based on the 2013 international Banff Classification Criteria (39) and defined as the presence of circulating donor-specific antibody and either: 1) peritubular capillary staining of C4d and at least one of the following: ptc score>0, g score>0, acute thrombotic microangiopathy in the absence of any other cause, or other features consistent with AMR (endothelial injury, fibrin thrombi, microinfarctions, interstitial hemorrhage), or 2) absence of capillary staining of C4d and the presence of ptc>0 and g>0 or ptc>0 or g>0 and acute thrombotic microangiopathy, in the absence of any other cause of thrombotic microangiopathy.

Clinical episodes of AMR were distinguished from subclinical episodes by the presence of graft dysfunction, manifested as oliguria/anuria, an increase in serum creatinine by $\geq 20\%$ from baseline, treatment of cell-mediated rejection and/or thrombotic microangiopathy within the two prior weeks, or new onset proteinuria.

CHAPTER 4 - Eculizumab and Splenectomy as Salvage Therapy for Severe Antibody-Mediated Rejection Following HLA-Incompatible Kidney Transplantation

Babak J. Orandi¹, Andrea A. Zachary², Nabil N. Dagher¹, Serena M. Bagnasco³, Jacqueline M. Garonzik-Wang¹, Kyle J. Van Arendonk¹, Natasha Gupta¹, Bonnie E. Lonze¹, Nada Alachkar², Edward S. Kraus², Niraj M. Desai¹, Jayme E. Locke⁴, Lorraine C. Racusen³, Dorry L. Segev¹, Robert A. Montgomery¹

Author Affiliations: Johns Hopkins Medical Institutions, Departments of Surgery¹, Medicine², and Pathology³, University of Alabama-Birmingham, Department of Surgery⁴

ABSTRACT:

Incompatible live donor kidney transplantation is associated with an increased rate of antibody-mediated rejection (AMR) and subsequent transplant glomerulopathy. For patients with severe, oliguric AMR, rapid graft loss is inevitable without timely intervention. We reviewed our experience rescuing kidneys with this AMR phenotype by using splenectomy alone (n=14), eculizumab alone (n=5), or splenectomy plus eculizumab (n=5). The study population was 267 consecutive patients undergoing desensitization for an HLA-incompatible live donor renal transplant. In the first 3 weeks after transplantation (mean=6 days), 24 patients developed sudden onset oliguria and/or rapidly rising serum creatinine with marked rebound of donor specific antibody, and a biopsy that showed features of severe AMR. At a median follow up of 533 days, 4 of 14 splenectomy alone patients experienced graft loss (median=320 days), compared to 4 of 5 eculizumab alone patients with graft failure (median=95 days). No patients treated with splenectomy plus eculizumab experienced graft loss. There was more chronic glomerulopathy in the splenectomy alone and eculizumab alone groups at 1 year, while splenectomy plus eculizumab patients had almost no transplant glomerulopathy. These data suggest that for patients manifesting early severe AMR, splenectomy plus eculizumab may be the preferred treatment

INTRODUCTION:

Sensitization to HLA is one of the most significant barriers to access to transplantation. For approximately 30,000 patients on the kidney transplant waiting list in the USA, sensitization will mean prolonged waiting times and a high wait list death rate (15). Live donor kidney transplantation, through the use of various desensitization protocols, has given highly sensitized patients the opportunity to experience the benefits of renal transplantation. However, patients who are desensitized do experience higher rates of antibody-mediated rejection (AMR) and outcomes are not as good as patients who receive HLA compatible kidneys (58). The main challenge facing the field of HLA desensitization is the high rate of chronic glomerulopathy (cg) associated with antibody-mediated injury and the resultant shortened allograft half-life (104, 105).

Early acute AMR, characterized by deposition of C4d in the peritubular capillaries (PTC) and peritubular neutrophil margination in the presence of donor specific antibody (DSA) occurs in 20-40 percent of patients after desensitization for an HLA-incompatible transplant (80, 102, 106). Early AMR is usually mild and responds to additional treatment with plasmapheresis (PP), intravenous immunoglobulin (IVIg), and anti-CD20 therapy (16, 41). However, about 5-10% of patients undergoing desensitization will experience a very strong DSA rebound and severe oliguric AMR that can be recalcitrant to standard therapy, rapidly resulting in cortical necrosis and graft loss [7]. Rescue splenectomy has been described as allograft salvage therapy for patients with this phenotype (107). Newly formed plasmablasts migrate to the spleen, and splenectomy can

result in a rapid reduction in DSA production. PP is then able to clear the remaining circulating antibody, and most kidneys will recover function. However, transplant glomerulopathy is accelerated in these grafts.

Eculizumab (Soliris, Alexion) is a humanized monoclonal antibody that binds the complement protein C5, preventing cleavage into its effector molecules C5a and C5b, the latter of which is required for generation of the membrane attack complex and cell lysis (108). We reasoned that the mechanism of action of this biologic agent might provide protection against antibody-mediated injury to the vascular endothelium preventing activation of the cascade that leads to irreversible microvascular coagulative necrosis. Blocking acute complement-mediated injury might then allow time for the DSA to be removed with aggressive PP therapy. Early AMR after desensitization is thought to be due to anamnestic B-cell activation and is complement-dependent in most cases.

We first used eculizumab in 2004 for patients with severe AMR that could not undergo splenectomy [8]. The results were mixed, probably because we had not done anything to slow the production of antibody from splenic plasmablasts and at high DSA levels, there was still injury occurring. We hypothesized that combining eculizumab with splenectomy and PP might allow these kidneys to be rescued and prevent the acute injury that results in transplant glomerulopathy. In this study we compare the outcomes of 24 patients with the severe oliguric AMR phenotype who were treated with either

splenectomy, eculizumab, or splenectomy combined with eculizumab, in addition to PP, IVIg, and anti-CD20.

METHODS:

Patient Population

This is a retrospective analysis of an approved prospective database which undergoes annual review by the Johns Hopkins Institutional Review Board. All patients were treated with a standardized treatment protocol. An Investigational New Drug application for off-label compassionate use of eculizumab was initially approved by the Food and Drug Administration for the indication of severe AMR in 2004.

Between October 2003 and June 2012, 267 patients underwent live donor kidney transplantation at Johns Hopkins and had detectable DSA prior to the initiation of desensitization (HLA incompatible). The study cohort consists of all patients who were recipients of HLA- or HLA- plus ABO-incompatible live donor kidney transplants and developed severe oliguric AMR within the first month after transplantation. We define this phenotype as consisting of sudden, early renal dysfunction with oliguria (urine output falling by > 50% in 24 hours) and/or a 50% rise in serum creatinine, with a concomitant increase in DSA and histology consistent with AMR (including features associated with severe AMR such as extensive endothelial injury, fibrin thrombi, micro-infarctions, interstitial hemorrhage), as well as C4d and margination of neutrophils in the PTC. All patients were treated with PP and low-dose IVIg during their AMR episode. Additionally,

the patients either received splenectomy, eculizumab, or both splenectomy and eculizumab. Splenectomy was performed emergently as soon as the diagnosis of severe AMR was confirmed. Clinical and biochemical data abstracted from the database, and pathologic data from renal allograft tissue obtained at the time of the AMR-defining episode and from protocol biopsies at 6 and 12 months were analyzed.

Eculizumab Administration

Eculizumab was given intravenously at a dose of 1200 mg on the day the diagnosis of AMR was confirmed. A dose of 900 mg was administered the next day. After each PP treatment for the AMR episode an additional dose of 600 mg of eculizumab was given. This dosing was based on the pharmacokinetic and pharmacodynamic studies from the index case in 2004 and confirmed in subsequent patients (26, 109). During PP and up to 1 month after the initial dose, patients also received a weekly dose of 900 mg. Beginning on week 5, doses of 1200 mg were administered every two weeks for patients who remained on the drug. Eculizumab was discontinued when either the DSA dropped below a strength that would yield a positive flow cytometric crossmatch (FCXM) (roughly 5,000 mean fluorescence intensity [MFI]) or AMR had resolved on biopsy.

Desensitization and immunosuppression

All patients received PP and DSA monitoring prior to the transplant and post-transplant by protocol as previously described (15). Every other day PP treatments were followed by the administration of IVIg (intravenous cytomegalovirus immune globulin, 100mg/kg,

Cytogam®, CSL Behring). 21 of 24 patients received at least 1 dose of anti-CD20 antibody (rituximab, 375 mg/m², Rituxan®, Genentech).

Mycophenolate mofetil (2 g/day) and tacrolimus (target serum level 8 to 12 ng/ml) were administered beginning with the first pre-transplant PP treatment. Induction immunosuppression therapy was achieved with either daclizumab (2mg/kg, Zenapax®, Roche) or antithymocyte globulin (1.5 mg/kg daily for 5 days, Thymoglobulin®, Genzyme). Methylprednisolone 500 mg was administered daily for three days beginning intraoperatively; corticosteroids were then tapered over five days, and converted to prednisone 30 mg daily. Prednisone was tapered over a three-month period to 5 mg daily.

Antibody testing

Crossmatch tests, including anti-human globulin-enhanced lymphocytotoxicity (AHG-CDC) with T cells, one wash CDC (1wCDC) with B cells, and flow cytometry with T and B cells were performed. If present, antibodies were characterized in tests of multianalyte beads bearing individual class I and/or class II phenotypes (LIFEMATCH-ID, Gen-Probe, Stamford, CT) and, when needed for confirmation, tests of multianalyte beads bearing single antigens (LABscreen Single Antigens, One Lambda, Canoga Park, CA). Results were read on a Luminex® fluoroanalyzer with results expressed as mean fluorescence intensity. Titers for DSA were based upon IgG antibodies.

Reactions with test beads yielding MFI ≥ 1000 were considered positive. Although the sensitivity of detection varies for different HLA antibodies, in general, positive flow cytometric crossmatch (FCXM) tests correlated with the following MFI values from the solid phase immunoassays: ≥ 5000 on phenotype panels and $\geq 10-15,000$ on single antigen panels. Positive complement-dependent cytotoxicity crossmatch (CDC) results were associated with $\geq 10,000$ MFI on phenotype panels. However, caution must be exercised when interpreting these results due to significant variation in the results of solid phase assays within and especially between laboratories and nothing replaces actually performing the crossmatch.

In most cases, sera were available from the pre-desensitization, post-transplantation nadir, and AMR samples, and they were retested since major changes in testing technology and methodology occurred over the period of the study. When donor cells were available actual crossmatch testing was performed, otherwise, the results represent virtual crossmatches.

Histology and Immunohistochemistry

C4d staining was performed on frozen tissue by indirect immunofluorescence using anti-human C4d antibody (Quidel, San Diego, CA) at 1:40 dilution, followed by secondary antibody (fluorescein-isothiocyanate-conjugated goat anti-mouse IgG) or on paraffin embedded tissue sections using a rabbit polyclonal anti-human antibody at a 1:50 dilution, coupled with a biotin free polymer detection system (Lecia).

Biopsies were evaluated for cell mediated rejection and AMR based on updated Banff '97 criteria (110, 111). C4d staining was considered positive if present in $\geq 50\%$ of the PTC with intensity $\geq 1+$ (C4d2-3). The Banff histological parameters were used to score biopsies for: tubulo-interstitial scarring as ci (interstitial fibrosis) + ct (tubular atrophy); presence of glomerulitis: $g \geq 1$; peritubular capillaritis: $ptc \geq 1$; and chronic transplant glomerulopathy: $cg \geq 1$.

Renal Function

Renal function was assessed as the serum creatinine nadir in the month following kidney transplantation. For patients who had functioning grafts at 1 year, the serum creatinine on the day of the 1-year allograft biopsy (or closest to that date) was used as the 1-year estimation of allograft function.

Statistical Analysis

Data were analyzed using Stata (Stata Statistical Software: Release 12. College Station, TX: StataCorp LP). Patient and graft survival were explored using Kaplan-Meier analysis and compared between groups using log-rank testing. Between-group comparisons were made using analysis of variance, Fisher's exact test, and Kruskal-Wallis tests, as appropriate. A p-value less than 0.05 was considered statistically significant.

RESULTS:

Patient Population

Of 267 HLA-incompatible kidney transplants performed during the study period, we identified 24 patients with early severe AMR (9.0%), 14 of whom received splenectomy alone as salvage therapy. Five received eculizumab alone, and another 5 received a combination of splenectomy and eculizumab. In addition to HLA-incompatibility, 1 splenectomy alone patient, 2 eculizumab alone patients, and 1 combination treatment patient were also ABO-incompatible with their donor. In only the combination therapy patient could the AMR be attributed to isohemagglutinin rebound rather than DSA.

Table 1 describes the summary patient characteristics according to therapy received. The majority of patients were female (58.3%) and white (91.7%). Fifteen patients (62.5%) had undergone prior transplantation, including 5 who had undergone more than one previous transplant. Median panel reactive antibody (PRA) was 97 (IQR: 85.5-100). The patients underwent a median of 3 (IQR 2-4.5) pre-transplant PP sessions and 12.5 (IQR 9.5-15) post-transplant sessions, including sessions to treat their index AMR episode. The median PRA for the splenectomy, eculizumab, and combined therapy groups were 99.5% (interquartile range [IQR]: 94-100), 97% (IQR: 85-97), and 86% (IQR: 0-94).

Table 2 provides more individualized information about each patient. Amongst the etiologies for end stage renal disease, congenital anomalies of the kidney and urinary tract, diabetes mellitus, focal segmental glomerulonephritis, and glomerulonephritis were the most common etiologies, occurring in 6, 5, 4, and 4 patients, respectively.

The beginning DSA strength for the 3 groups was not the same. For the splenectomy, eculizumab, and combined treatment groups, 64%, 40%, and 0% started desensitization therapy with a positive CDC crossmatch, 29%, 60% and 60% had a positive FCXM, and 7%, 0%, and 40% had DSA below the FCXM threshold but detectable by Luminex testing, respectively.

However, Table 2 shows that the change in DSA strength between the groups from the post-transplant nadir to the AMR defining episode was not different. In fact, the patients in the combined treatment group increased from -FCXM strength to a + CDC crossmatch representing a very large rebound in DSA. Thus, the better outcomes and lower rates of transplant glomerulopathy in this group cannot be explained by a lower pre-desensitization DSA level.

Post-Transplantation Events

Patients were diagnosed with AMR at a median of 6 days post-transplant (IQR 5-9) and, of those undergoing splenectomy, the operation was performed at a median of 7 days post-transplantation (interquartile range 6-9) (Table 1). The median number of eculizumab doses was 12.5 (interquartile range 4-17). Of the 14 splenectomy alone patients, 4 lost their grafts at a median of 320 days post-transplant (IQR: 62-538). Four eculizumab alone patients experienced graft loss at a median of 95 days (IQR: 44.5-138). One-year death-censored graft survival was 77.9% in the splenectomy alone group, 30.0% in the eculizumab alone group, and 100.0% in the combination treatment group (P=0.07) (Figure 1).

Renal Function

At the time of AMR diagnosis, the median serum creatinine (mg/dL) was 2.9 (IQR: 2.3-3.6), 4.6 (IQR: 4.4-5.3), and 2.1 (IQR: 2.0-2.8) for the splenectomy, eculizumab and combination treatment groups, respectively (Figure 2). By 1 year, the median serum creatinine was 1.9 (IQR: 1.5-2.2) for the splenectomy group. The single patient with a functioning graft at 1 year in the eculizumab group had a creatinine of 1.8 mg/dL. The median serum creatinine in the combination treatment group at 1 year was 1.6 (IQR: 1.2-2.1).

Representative profiles from patients in each treatment group

Figure 3 demonstrates the changes in serum creatinine and urine output over the course of AMR treatment as well as a detailed timeline of peri-transplant antibody-lowering therapies for a representative patient from each treatment group. Figure 3a depicts a patient who had a sudden onset of anuria and a sharp rise in serum creatinine on post-transplantation day (PTD) #5 preceded 1 day by the appearance of new class I DSA (A2, B7) and a rise in class II DSA (DR15/51) to a +FCXM strength. The diagnosis of AMR was confirmed on PTD # 5 on biopsy, and the splenectomy was performed the same day. The patient remained anuric for 10 days and was on dialysis before renal function recovered with a return of urine output followed by a fall in serum creatinine. Coincident with the return of urine output the class I and II DSA dropped below the threshold for a positive Luminex assay. The patient's creatinine returned to 1.2 mg/dL but she

developed early transplant glomerulopathy and on PTD #340 she lost her graft during an episode of ischemic colitis.

Patient 3b received eculizumab in addition to PP/IVIg and anti-CD20. This was a pre-emptive transplant and the patient had normal urine output pre-transplantation.

Beginning on PTD #3 her creatinine began to increase and her class I DSA (A2, B35, B44) rose sharply. A biopsy on PTD #4 confirmed early AMR. The creatinine stabilized between PTD #4-6 with daily PP but then began to rise rapidly and on PTD #7 eculizumab was begun. DSA continued to increase and peaked on PTD #10. The kidney never regained function and ruptured on PTD #23 requiring emergent transplant nephrectomy. The explant showed severe AMR with cortical necrosis and interstitial hemorrhage.

Splenectomy and eculizumab were used to treat patient 3c in addition to PP/IVIg and anti-CD20. The patient's creatinine began to rise on PTD #6. DSA to A24, DR11, DR52, and DP began to acutely rebound on PTD #4 and then fell on PTD #7 after eculizumab was started and a splenectomy was performed. A return of urine output began within hours of the splenectomy and at 1 month the serum creatinine was 1.0 mg/dL. At 1 year the patient's cg score was 0.

Transplant glomerulopathy and surveillance biopsies

As expected, chronic glomerulopathy (cg) was absent at the time of the AMR-defining biopsy. Figure 4 demonstrates rates of graft loss, missing data, and the evolution of

transplant glomerulopathy by treatment group over the year following transplantation. In the splenectomy-alone group at 6 months, there were 3 graft losses and 1 patient did not have a biopsy. 1/10 (10%) of patients had a cg score of 2 and 1/10 (10%) had a cg score of 3. The remaining 8/10 (80%) had a cg score of 0. There were 2 immediate graft losses in the eculizumab-alone group. Of the 3 that made it out to 6 months, 1 patient (33.3%) had a cg score of 2, 1 had a score of 1 (33.3%), and 1 had a cg score of 0 (33.3%). In the combination treatment group, there were no graft losses or missing data, 1 patient had a cg score of 1 (20.0%), and the remaining 4 (80.0%) had a cg score of 0.

At 12 months, in the splenectomy-alone group there were 4 graft losses and 1 missing data point. 2/9 patients (22.2%) had a cg score of ≥ 2 , 3/9 (33.3%) had a score of 1, and 4/9 (44.4%) had a score of 0. The remaining eculizumab-alone patient with a functioning graft at 1 year had a cg score of 2. In the combination treatment group, 1 patient (20.0%) had a cg score of 1, and the remaining 4/5 (80.0%) had a score of 0. In summary, in the splenectomy-alone group only 29% of the patients had functioning allografts and a 1-year biopsy free of transplant glomerulopathy. In the eculizumab-alone cohort all grafts were either lost or had transplant glomerulopathy. In the combined group 80% of the patients had functioning grafts free of transplant glomerulopathy.

Figure 5 demonstrates the pathologic findings on the AMR-defining biopsy and on subsequent biopsies at either 6 months or 1 year in a representative patient from each treatment group. The patient in the splenectomy-alone group had severe AMR on her index biopsy characterized by diffuse C4d staining with marginating neutrophils and

infarcted glomeruli (panels A,B,C). The 6 month biopsy on this patient showed widening of the subendothelial space and cytoplasmic interposition on electron microscopy (D).

Glomerular double contours are present on the 1 year biopsy (E).

The second panel is from the patient in the eculizumab-alone group. The AMR-defining biopsy shows extensive interstitial hemorrhage (F) minimal glomerular damage (G) and C4d positivity (H). The 1-year biopsy demonstrates widening of the subendothelial space and cytoplasmic interposition on electron microscopy (I) and an ischemic glomerulus (J).

Panel 3 is from the representative patient in the combination treatment group. The first 3 panels from a biopsy at the time of the AMR diagnosis show PTC margination (K), a glomerulus with fibrin deposits and thrombotic microangiopathy (L), and positive C4d immunofluorescence staining (M). A biopsy at 1 year has a relatively normal-appearing glomerulus without double contours (N, O).

DISCUSSION

For patients undergoing desensitization prior to live donor kidney transplantation, graft loss within the first month from severe AMR remains one of the most off-putting realities facing programs considering taking on patients with heightened immunologic risk from HLA sensitization. Not only are these losses devastating on a human level, but regulatory benchmarking that does not account for this increased risk liability raises the specter of Centers for Medicare & Medicaid flagging and decertification. In our experience from desensitizing 267 patients with DSA, this AMR phenotype occurs at an

alarming rate of 9%. We have previously shown that daily PP, low dose IVIg, and anti-CD20 are not effective in rescuing these kidneys, and graft loss is almost universal (107). This is likely because the strength of the anamnestic responses that produce this phenotype overwhelms the capacity of PP to keep pace with new antibody production, and anti-CD20 is not effective against plasmablasts that are responsible for this rapid antibody release. We, and others, reported that immediate splenectomy could rescue most kidneys undergoing severe, early oliguric AMR (112). Plasmablasts traffic from regional lymph tissue to the spleen before maturing and taking up residence in the bone marrow where they are long-lived and protected in a niche microenvironment. The plasmablasts are vulnerable to debulking by virtue of the enrichment of these cells in the spleen at the time of a severe AMR. Indeed, spleens explanted from these patients are packed with donor-specific plasmablasts and plasma cells (113-115).

While splenectomy has been effective at decreasing rates of early graft loss to acceptable levels for patients and regulatory organizations, we have observed in this study that allografts rescued by splenectomy have a high rate of transplant glomerulopathy within a year of the AMR episode and a steady attrition. Splenectomy appears to produce source control of new antibody production but high levels of soluble antibody must still be cleared by PP and this takes days to accomplish. During this time the renal endothelium is subjected to unbridled antibody-driven, complement-mediated cytolysis. Complement split products amplify injury through chemotactic signals and recruitment of effector cells. The lesions that result in transplant glomerulopathy appear to start during the acute AMR episode and in most cases are not due to ongoing antibody injury since PP in this study

was continued until DSA was eliminated or reduced below a positive FCXM strength. A mechanistic pathway-driven therapeutic approach, as well as early success in reversing AMR in small animal experimental models (116) using complement inhibition prompted us to first use eculizumab in a patient experiencing severe AMR in 2004 (26). We were also able to work out the pharmacokinetics of using eculizumab concurrently with PP, which we think is essential.

In this study, we report the results of three approaches to reversing severe, early AMR after desensitization using splenectomy, eculizumab, and a combination of both interventions. In addition to one of these 3 treatment modalities, all patients were treated with PP and low dose IVIg and most received anti-CD20. Splenectomy produced a salvage rate of 86% at PTD #60, but by 1 year, only a third of the kidneys were still functioning and free from transplant glomerulopathy. Eculizumab without splenectomy was not effective in this small cohort and all 5 patients treated either lost their kidney or developed transplant glomerulopathy. Combining splenectomy and eculizumab produced the best results with 100% rescue and graft survival at 1 year. On 1-year protocol biopsies, only 1 patient out of 5 was found to have a cg >0. Pre-desensitization strength differed between the treatment groups with the combination treatment group starting off with the lowest level DSA. However, this did not prevent patients in this group from developing severe AMR, and the actual change in antibody strength from the post-transplant nadir to the AMR episode was as great in the combined group as the other two cohorts, suggesting similarly robust anamnestic responses.

An attractive mechanistic model for explaining the success of the combined intervention is that splenectomy removes antibody-producing cells and eculizumab protects the endothelium from injury while PP is clearing residual circulating antibody. In other words, the splenectomy increases rescue rates, and the eculizumab prevents transplant glomerulopathy. The protective effect of eculizumab may not be durable and if strong DSA is allowed to persist for long periods of time chronic glomerulopathy develops (27). For this reason we believe it is important, especially when DSA strength is high as in these early AMRs, to deplete the antibody with PP until the FCXM is negative. Unfortunately, this will also remove eculizumab, and a dose of 600 mg of this costly drug must be given after each plasma exchange.

To date there are only case reports of using eculizumab to reverse AMR in allografts (26, 117-120). The Mayo group used eculizumab prophylactically in patients undergoing desensitization (27). They found a significant reduction in AMR, including the phenotype described in our study, during the first 3 months after renal transplantation. They did not perform post-transplantation PP, and DSA persisted in many of the patients in the absence of renal dysfunction and histologic evidence of AMR. However, some of these patients did go on to develop transplant glomerulopathy after the drug was withdrawn, and we think this supports our therapeutic design and mechanistic explanation for each component in the protocol described here. Also, since it has not been possible to predict which patients will develop early AMR, prophylactic use of eculizumab means treating all of the patients who are desensitized, when only 9% of them will develop graft-threatening AMR. It is unclear at this point whether the

additional cost of the drug will allow its use in the 91% of patients who will not develop severe AMR.

The limitations of this study are due primarily to the nonrandomized methodology and the fact that significant changes in technology and best practices occurred over the study period. However, the desensitization protocol remained consistent throughout, and changes in DSA testing were mitigated in part by retesting the serum from earlier time periods using current techniques. Additionally, the small sample size and the inability to adjust for important confounders limits our ability to draw definitive conclusions about the best treatment for severe AMR following HLA-incompatible live donor kidney transplantation.

In conclusion, these preliminary data suggest that splenectomy is effective at graft salvage, but long-term graft survival is poor due primarily to transplant glomerulopathy. Eculizumab alone does not appear to be sufficient to rescue kidneys when very high levels of DSA are present, but further work needs to be done to determine its efficacy for reversing AMR at lower antibody strengths. When administered in concert with splenectomy, eculizumab may offer the best opportunity for graft salvage and longer-term graft survival in patients with the most severe, imminently graft-threatening presentation of AMR; however, caution must be exercised because of the invasive nature of splenectomy and the significant cost of eculizumab.

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The authors of this manuscript have the following conflicts of interest to disclose: RAM has received a research grant from Alexion for a small clinical trial to study the efficacy of eculizumab in preventing catastrophic antiphospholipid antibody syndrome. RAM is also a co-investigator on a multi-center, international trial of eculizumab to prevent antibody-mediated rejection, funded by Alexion. Eculizumab was provided for the first two patients in the study by Alexion.

Table 1. Patient Characteristics by Treatment Received

	Overall (n=24)	Splenectomy Alone (n=14)	Eculizumab Alone (n=5)	Splenectomy and Eculizumab (n=5)	P-Value
Age at Transplant (SD)	43.7 (14.3)	48.3 (12.4)	27 (4.7)	47.6 (14.6)	0.89
Female	14 (58.3%)	9 (64.3%)	1 (20.0%)	4 (80.0%)	0.12
Black Race	2 (8.3%)	1 (7.1%)	0 (0.0%)	1 (20.0%)	0.67
Median PRA (IQR)	97 (85.5-100)	99.5 (94-100)	97 (85-97)	86 (74-94)	0.17
History of Prior Transplantation	15 (62.5%)	7 (50.0%)	4 (80.0%)	4 (80.0%)	0.31
DSA Strength Prior to Desensitization					0.06
Luminex+	3 (12.5%)	1 (7.1%)	0 (0.0%)	2 (40.0%)	
FCXM+	10 (41.7%)	4 (28.6%)	3 (60.0%)	3 (60.0%)	
CDC+	11 (45.8%)	9 (64.3%)	2 (40.0%)	0 (0.0%)	
DSA Strength at the Time of AMR		* †		§	0.53
Luminex+	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
FCXM+	6 (30.0%)	4 (36.4%)	2 (40.0%)	0 (0.0%)	
CDC+	14 (70.0%)	7 (63.6%)	3 (60.0%)	4 (100.0%)	
Induction Agent					
Anti-Thymocyte Globulin	12 (50.0%)	4 (28.6%)	4 (80.0%)	4 (80.0%)	0.02
Daclizumab	11 (45.8%)	10 (71.4%)	1 (20.0%)	0 (0.0%)	
Basiliximab	1 (4.2%)	0 (0.0%)	0 (0.0%)	1 (20.0%)	
Median Pre- Transplant PP Sessions (IQR)	3 (2-4.5)	3 (3-4)	4 (2-5)	3 (2-3)	0.78
Median Post- Transplant PP Sessions (IQR)	12.5 (9.5-15)	12 (8-13)	14 (10-15)	21 (15-24)	0.10
Days Until AMR Diagnosis (IQR)	6 (5-9)	5.5 (4-7)	6 (6-10)	9 (7-16)	0.06
Days From Transplant Until Splenectomy (IQR)	7 (6-9)	6 (5-8)	--	8 (8-11)	0.08

SD=standard deviation, PRA=panel reactive antibody, IQR=interquartile range, PP=plasmapheresis; CDC+ = complement-dependent cytotoxicity crossmatch positive, FCXM+ = flow cytometric crossmatch positive, Luminex+ = negative FCXM but DSA with mean fluorescence intensity > 1,000 on Luminex platform

* 2 patients did not have a crossmatch available at the time of AMR diagnosis.

† 1 patient had a negative FCXM, but new anti-B50 antibody.

§ 1 patient had no detectable donor-specific antibody, but a rebound of anti-A antibody (titer 32).

Table 2. Sensitization, Donor-Specific Antibody Strength, and Treatment of Antibody-Mediated Rejection

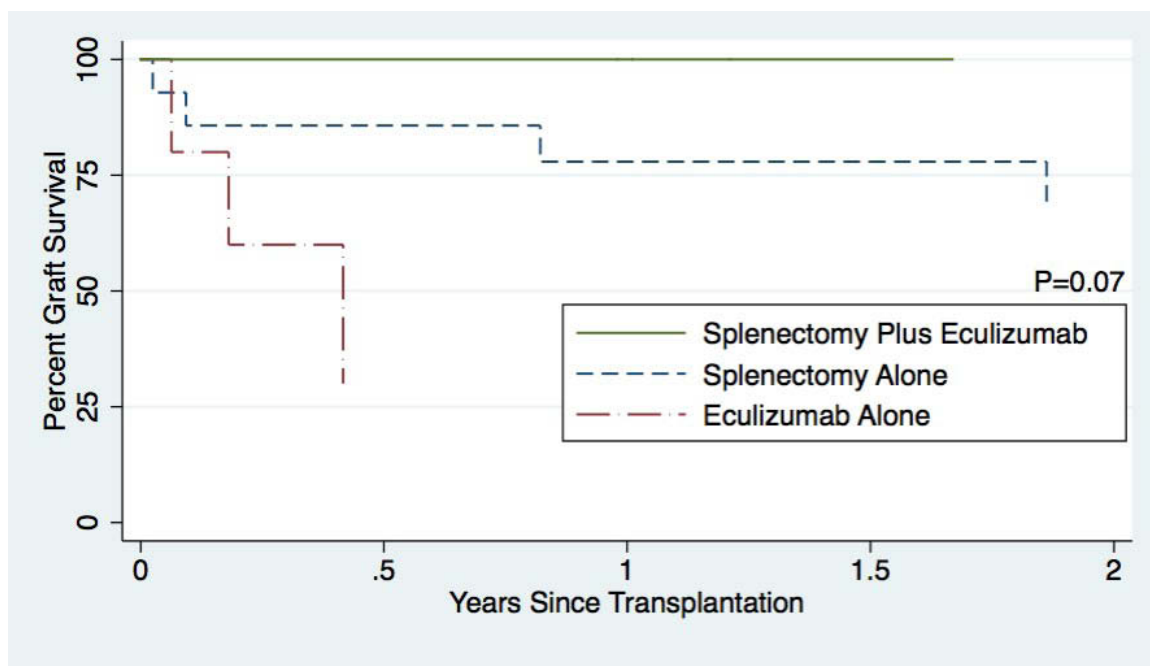
	Age/ Sex	Prior Transplant	PR A	DSA Strength Prior to Desensitization	DSA Strength at Post- Transplant Nadir	DSA Strength at the Time of AMR Diagnosis	Days Until AMR	Rituxima b Received	Number of Eculizumab Doses		
									120 0 mg	900 mg	600 mg
Splenectomy Alone											
1	51 F	-	95	CDC+	NA	NA	2	N	--	--	--
2	72 F	-	62	CDC+	No DSA	+FCXM	4	N	--	--	--
3	46 M	Kidney	98	FCXM+	NA	NA	7	Y	--	--	--
4	36 M	Kidney x 2	100	CDC+	+Luminex (1,600 MFI)	-FCXM (new anti- B50)	4	Y	--	--	--
5	27 M	Kidney	94	CDC+	No DSA	+CDC	9	Y	--	--	--
6	39 F	Kidney- pancreas	100	CDC+	No DSA	+CDC	6	Y	--	--	--
7	57 F	-	100	CDC+	+Luminex (1,700 MFI)	+FCXM (new anti- A2/B7)	5	Y	--	--	--
8	61 M	-	36	Luminex+	No DSA	+FCXM	6	Y	--	--	--
9	52 F	-	100	CDC+	+CDC (titer 1)	+CDC (titer 128)	1	Y	--	--	--
10	44 F	-	99	FCXM+	+Luminex (2,000 MFI)	+CDC	5	Y	--	--	--
11	47 M	Kidney	100	FCXM+	NT	+CDC	6	N	--	--	--
12	64 F	Kidney	100	CDC+	No DSA	+CDC (titer 16)	13	Y	--	--	--
13	34 F	Kidney	86	FCXM+	No DSA	+FCXM	5	Y	--	--	--
14	46 F	-	100	CDC+	+Luminex (1,500 MFI)	+CDC	8	Y	--	--	--
Eculizumab Alone											
15	25 M	Kidney x 2	97	FCXM+	+Luminex (1,100 MFI)	+FCXM	6	Y	3	1	0
16	32 F	-	48	CDC+	+Luminex	+FCXM	4	Y	1	2	9
17	30 M	Kidney	85	CDC+	+CDC	+CDC	11	Y	2	0	2
18	20 M	Kidney x 2	99	FCXM+	No DSA	+CDC (titer 8)	10	Y	0	0	1
19	28 M	Kidney	97	FCXM+	+Luminex	+CDC	6	Y	3	3	9
Splenectomy + Eculizumab											
20	26 F	Kidney	100	FCXM+	+Luminex (6,000 MFI)	+CDC	16	Y	2	4	26
21	52 F	Kidney	74	FCXM+	No DSA	+CDC	17	Y	1	1	5
22	41 F	Pancreas	86	FCXM+	+Luminex (2,700 MFI)	+CDC	6	Y	1	6	16
23	64 F	-	94	Luminex+	+Luminex (2,000 MFI)	+CDC	7	Y	1	6	10

24	55 M	Kidney, pancreas x 2	29	Luminex+, Anti-A titer 512	No DSA Anti-A titer 8	No DSA Anti-A titer 32	9	N	1	3	9
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Most patients had multiple DSA, but Luminex MFI listed are for the strongest DSA.

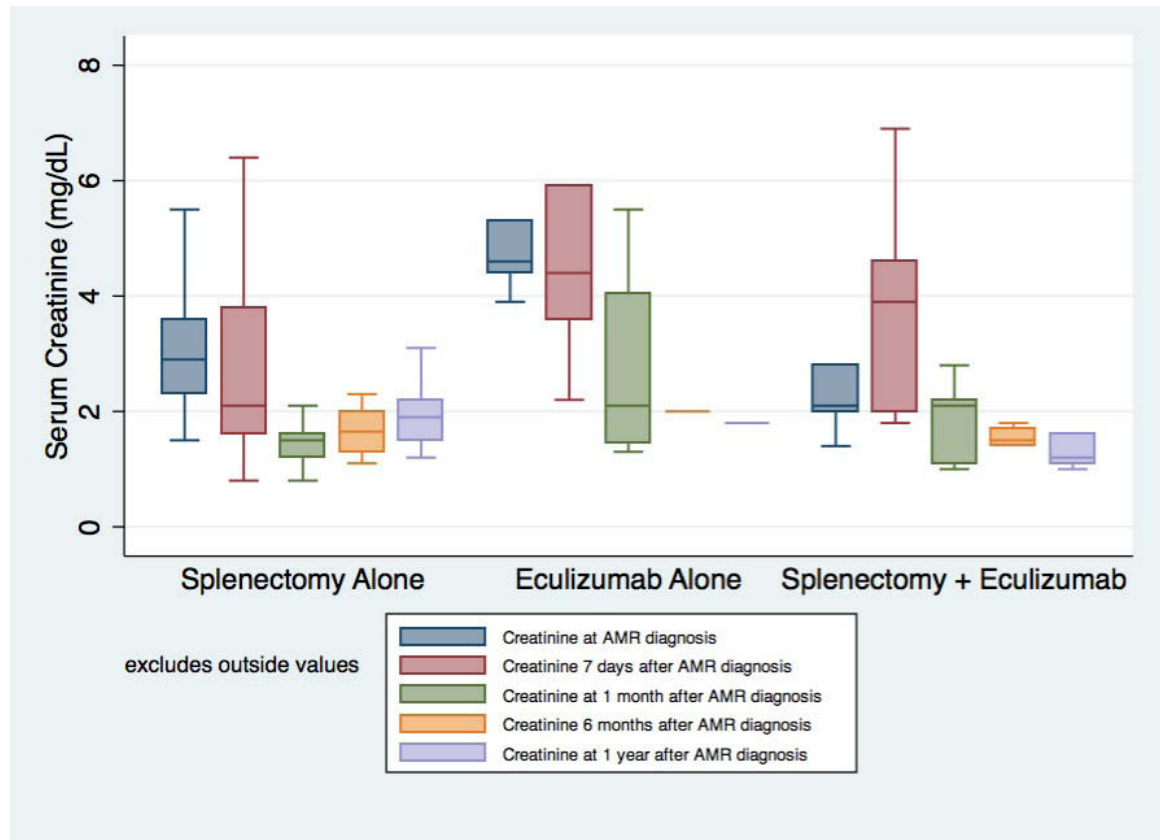
PRA = panel reactive antibody, AMR = antibody-mediated rejection, CDC+ = complement-dependent cytotoxicity crossmatch positive, FCXM+ = flow cytometric crossmatch positive, Luminex+ = negative FCXM but DSA with MFI > 1,000 on Luminex platform, MFI=mean fluorescence intensity, NA=not available (serum not available for retesting), NT=not tested

Figure 1. Death-censored graft survival by treatment received



AMR=antibody-mediated rejection

Figure 2. Post-transplant renal function over time by treatment received



AMR=antibody-mediated rejection

FIGURE 3. Serum creatinine, urine output, antibody levels, and details of peri-transplant events in a representative patient from the splenectomy alone group (A), the eculizumab alone group (B), and the combination treatment group (C).

The horizontal axis is relative to day of transplant (day 0), with the first date shown being the first day of desensitization therapy. The vertical yellow line represents the day that the AMR-defining biopsy was performed. AMR=antibody-mediated rejection, PP/IVIg=plasmapheresis and intravenous immunoglobulin, anti-CD20=anti-CD20 antibody (rituximab).

Figure 3a. Splenectomy Alone

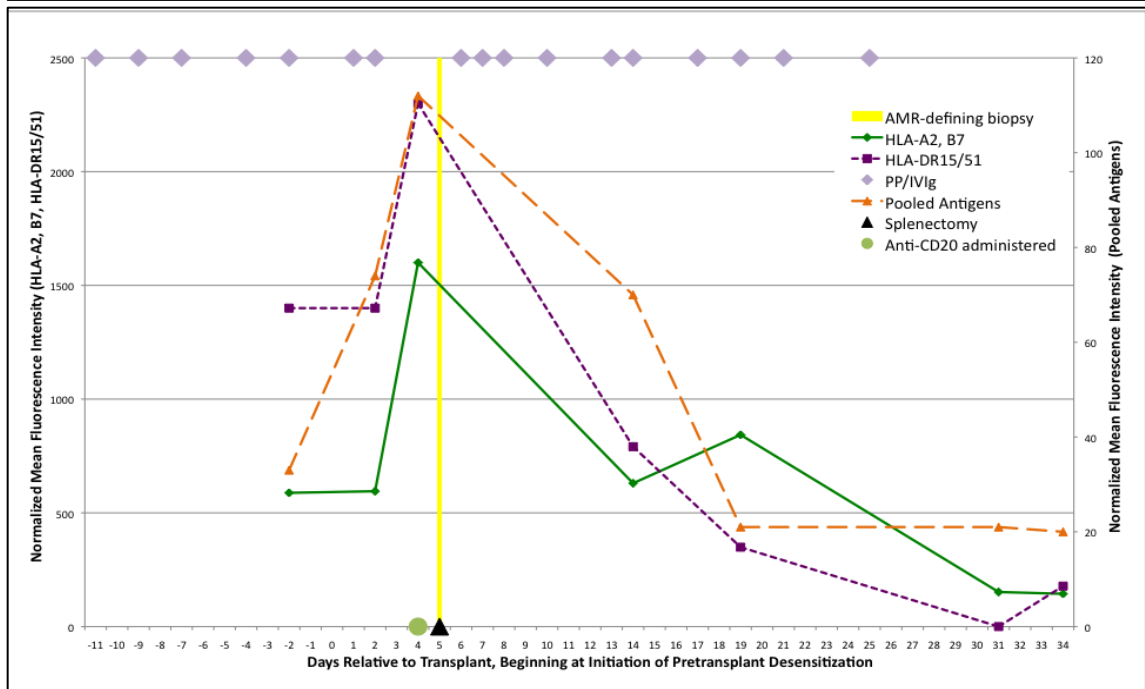
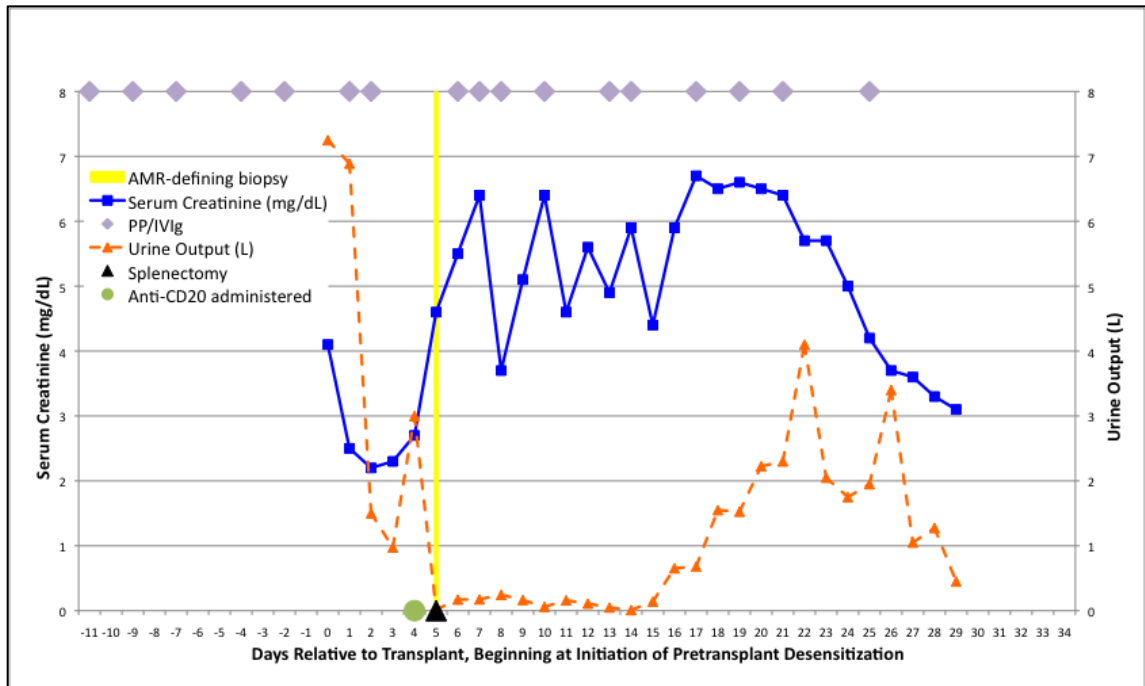


Figure 3b. Eculizumab Alone

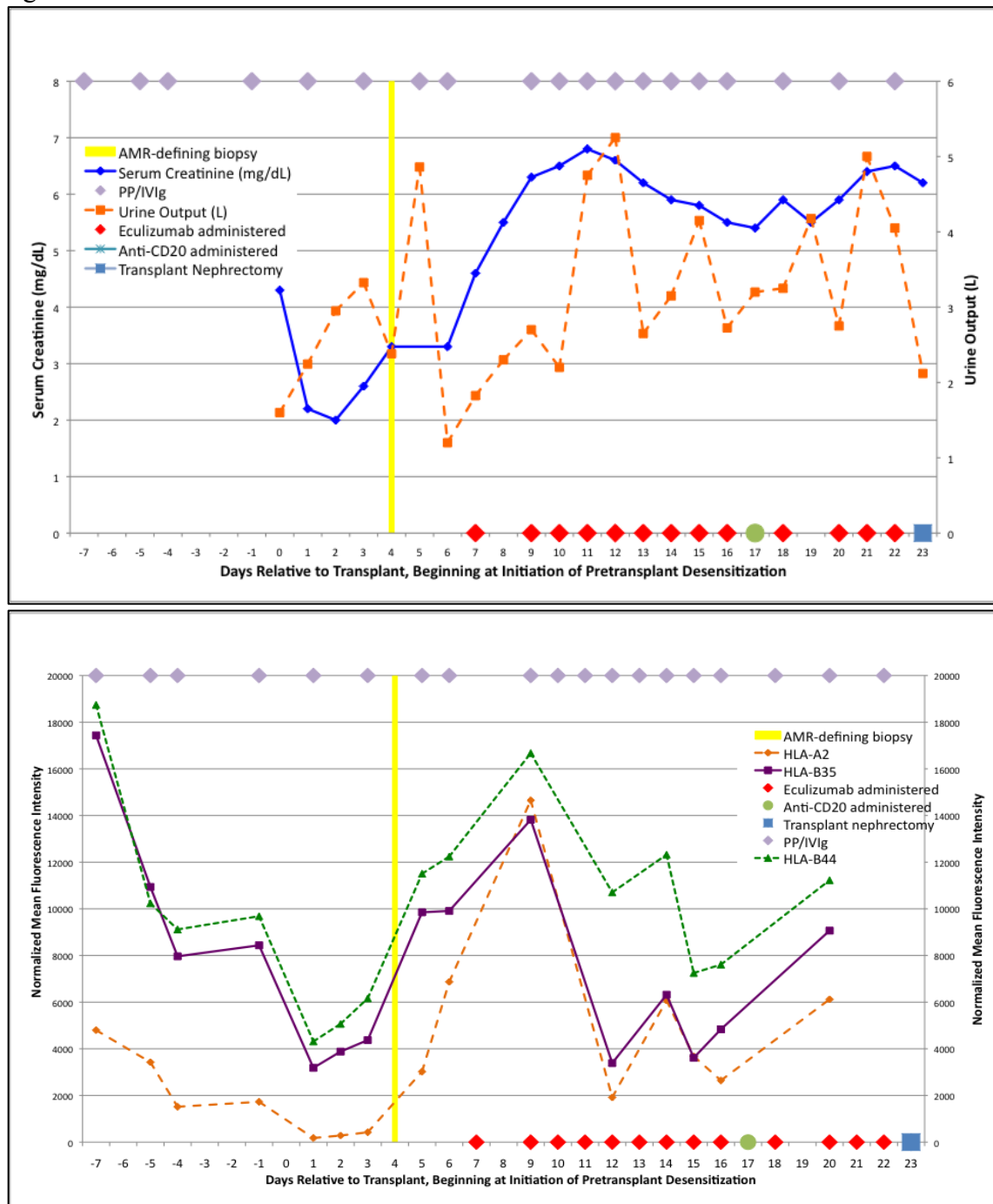


Figure 3c. Combination Treatment

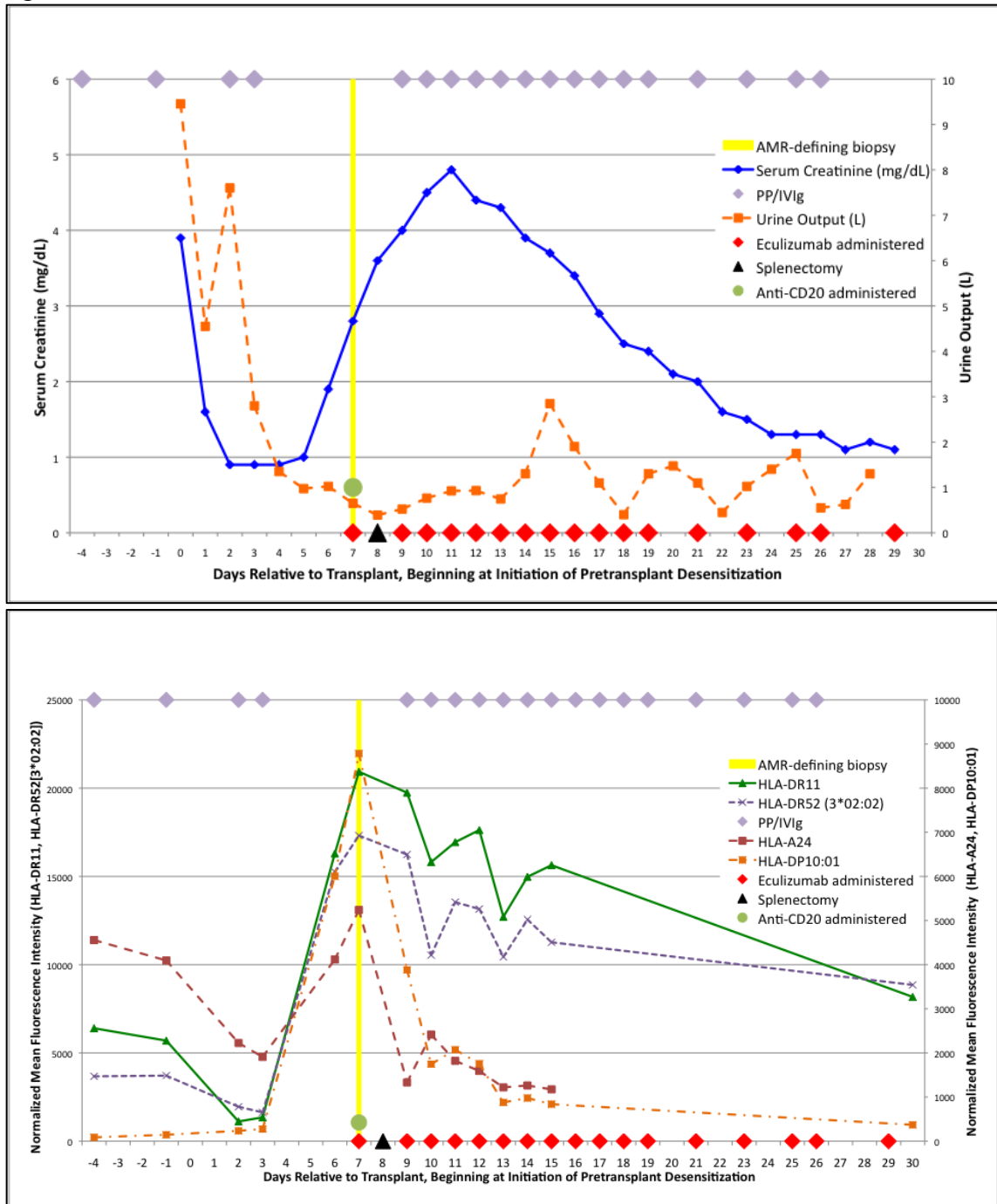
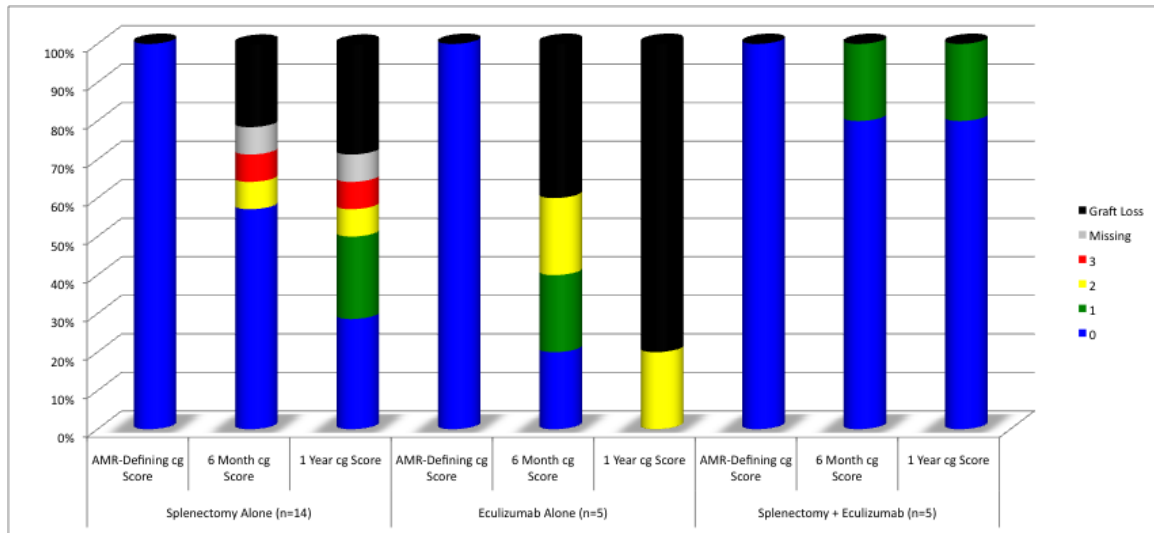


Figure 4. Banff chronic glomerulopathy score at the time of antibody-mediated rejection-defining biopsy, 6 months, and 12 months post-transplant by treatment received



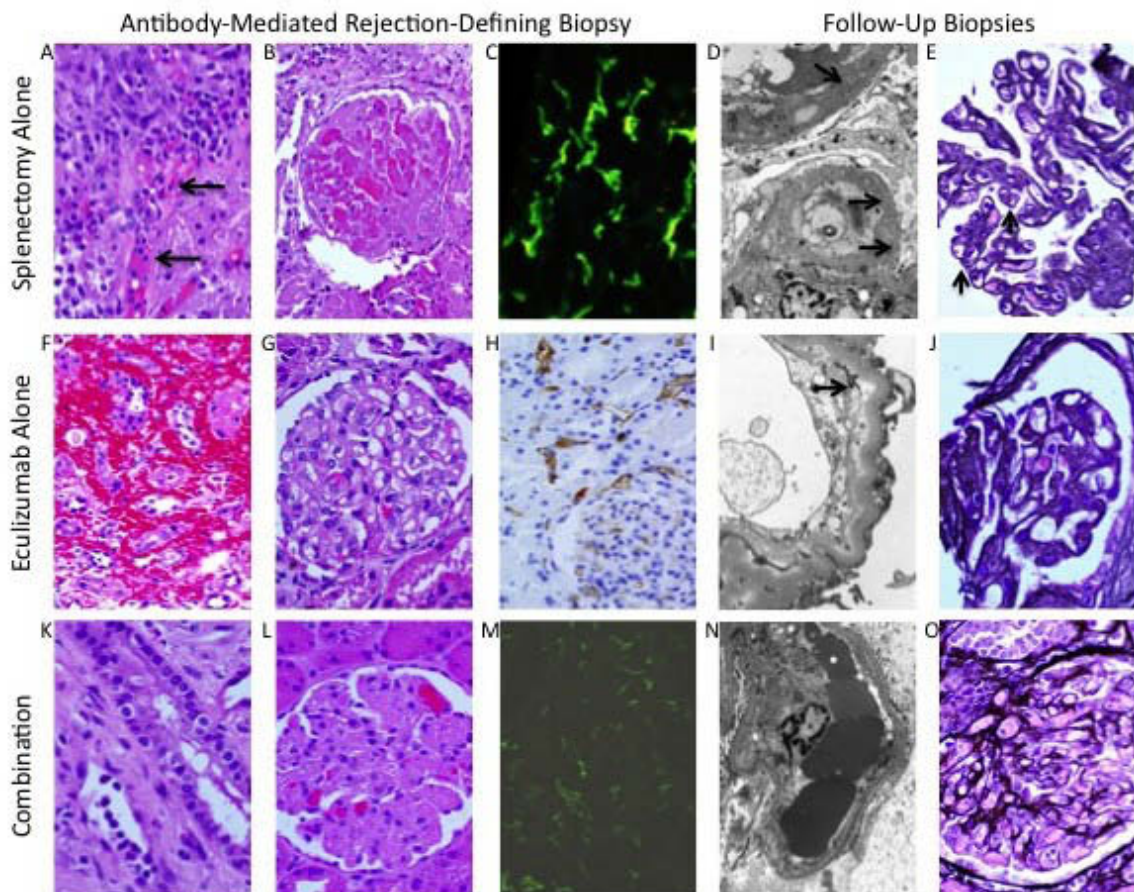
AMR=antibody-mediated rejection; cg=Banff chronic glomerulopathy score

Figure 5. Pathologic findings on the antibody-mediated rejection-defining biopsy and subsequent biopsies in a patient representative of each treatment group.

Splenectomy Alone Group: Antibody-mediated rejection-defining biopsy demonstrating (A) peritubular capillaries with marginating neutrophils (arrows; H&E, 600x), glomerular infarction (B; H&E, 400x), and positive C4d immunofluorescence staining (C). Electron microscopy at 6 months with arrows indicating widening of the subendothelial space and cytoplasmic interposition (D). Biopsy at 1 year demonstrating double counters (arrows) of the glomerulus and a cg score of 3 (PAS-MS stain, 600x).

Eculizumab Alone Group: Antibody-mediated rejection-defining biopsy demonstrating interstitial hemorrhage (F; H&E, 400x), minimal glomerular damage (G; H&E, 600x), and C4d positivity (H; immunohistochemistry, 400x). Biopsy at 1 year demonstrating widening of the subendothelial space and cytoplasmic interposition (I; arrow; electron microscopy) and an ischemic glomerulus with a cg score of 2 (J; PAS-MS, 600x).

Combination Treatment Group: Antibody-mediated rejection-defining biopsy that reveals peritubular capillary margination (K; H&E, 600x), a glomerulus with thrombotic microangiopathy (L; H&E, 600x) and positive C4d immunofluorescence staining (M). Biopsy at 1 year demonstrating relatively normal glomerulus (N; electron microscopy) and a borderline cg score of 0-1 (O; PAS-MS, 600x).



CHAPTER 5 - Conclusion

This work has examined several challenges associated with kidney transplantation in immunologically high-risk patients. First, the risks of graft loss and mortality for incompatible live donor kidney transplant (ILDKT) recipients have never been previously quantified precisely or in a multi-center fashion. Compared to compatible recipients, patients who were positive Luminex, negative flow crossmatch (PLNF) had a similar risk of graft loss; however, positive flow, negative cytotoxic crossmatch (PFNC) patients (aHR=1.64, 95% CI: 1.15-2.23, P=0.007) and positive cytotoxic crossmatch (PCC) patients (aHR=5.01, 95% CI: 3.71-6.77, P<0.001) had increased graft loss in the first year. Interestingly, the risk of graft loss attenuated somewhat for PCC patients after the first year, but continued to remain elevated compared to compatible recipients (aHR=1.80; 95% CI: 1.42-2.29; P<0.001). As with graft loss, PLNF patients had a similar risk of death compared to compatible patients; however, PFNC (aHR=2.04; 95% CI: 1.28-3.26; P=0.003) and PCC transplantation (aHR=4.59; 95% CI: 2.98-7.07; P<0.001) were associated with increased mortality. PCC recipients saw a similar attenuation in the increased risk of death after the first year post-transplant as they did with graft loss (aHR=1.51; 95% CI: 1.13-2.03; P<0.001). These findings were then used to quantify the risk of flagging by the Centers for Medicare and Medicaid Studies (CMS) for regulatory scrutiny. Compared to equal-quality centers performing no ILDKT, centers performing 5%, 10%, or 20% PFNC had a 1.19, 1.33, and 1.73-fold higher odds of flagging. Centers performing 5%, 10%, or 20% PCC had a 2.22, 4.09, and 10.72-fold higher odds of CMS flagging.

ILDKT patients are at particularly high risk of developing antibody-mediated rejection (AMR), and it has been unclear in the literature if AMR differentially affects graft survival by transplant phenotype (39). Examining the Johns Hopkins Hospital transplant population and using a robust, up-to-date definition of AMR, we formed the largest cohort in existence of AMR patients. We demonstrated that the incidence of AMR varies dramatically by transplant phenotype. Compatible live donor recipients have an AMR incidence of 0.7%, but live donor HLA-incompatible recipients have an incidence of 45.1%. The risk of graft loss appears to vary by phenotype as well. While live donor ABO-incompatible recipients do not appear to have an increased risk of graft loss, live donor HLA-incompatible recipients have a 6.29-fold increase in the risk of graft loss (95% CI: 3.81-10.39; $P < 0.001$).

Finally, in studying AMR in ILDKT patients, it became clear that there exists a particularly severe form of AMR that rapidly leads to graft loss without timely and aggressive intervention. In examining the Hopkins experience with early, severe AMR, we identified three different approaches to graft salvage, in addition to the standard of care of plasmapheresis, intravenous immunoglobulin, and anti-CD20 therapy--splenectomy, eculizumab, and a combination of both interventions. Splenectomy alone produced a reasonable immediate salvage rate of 86% at post-transplant day#60, but by 1 year, only a third of the kidneys were still functioning and free from transplant glomerulopathy. Eculizumab without splenectomy was not effective in this small cohort, and all 5 patients treated either lost their graft or developed transplant glomerulopathy.

Combining splenectomy and eculizumab produced the best results with 100% rescue and graft survival at 1 year. On 1-year protocol biopsies, only 1 patient out of 5 was found to have transplant glomerulopathy.

These results have spurred a number of new scientific questions, and the development of these study cohorts has allowed us to begin to answer some of these questions. For example, linking the ILDKT multi-center cohort to Medicare claims data will allow us to answer a number of important questions about outcomes of ILDKT patients in terms of infectious complications and the development of malignancies. The AMR cohort will enable us to quantify the outcomes of patients who develop concurrent AMR and various types of T-cell-mediated rejection and explore for differences in graft outcomes between patients developing C4d-positive AMR and C4d-negative AMR. Despite the many clinical and regulatory challenges that remain in optimizing the care of ILDKT patients, ILDKT is an exciting field on the cutting edge of science and clinical care. More importantly, it offers patients improved survival and the possibility of a life free from dialysis.

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105. Issa N, Cosio FG, Gloor JM, Sethi S, Dean PG, Moore SB, et al. Transplant glomerulopathy: risk and prognosis related to anti-human leukocyte antigen class II antibody levels. *Transplantation*. 2008;86(5):681-5.
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111. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2008;8(4):753-60.
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114. Kaplan B, Jie T, Diana R, Renz J, Whinery A, Stubbs N, et al. Histopathology and immunophenotype of the spleen during acute antibody-mediated rejection. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2010;10(5):1316-20.
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120. Hamer R KN, Lowe D, Briggs D, Zehnder D, Higgins R. C5b-9 inhibitor (eculizumab) for antibody-mediated rejection in renal transplantation. *Indian J Transplant*. 2011;5(1):6-8.

CURRICULUM VITAE

29 March 2014

Babak J. Orandi, MD MSc

Doctoral Candidate, Johns Hopkins Bloomberg School of Public Health
Graduate Training Program in Clinical Investigation
Halsted Resident, Johns Hopkins University School of Medicine, Department of Surgery

PERSONAL DATA

Babak J. Orandi, MD MSc
The Johns Hopkins University School of Medicine
Department of Surgery
32 Turner
720 Rutland Avenue
Baltimore, MD 21297
Phone: (734) 717-2759
Email: borandi1@jhmi.edu

POST-GRADUATE MEDICAL TRAINING/EMPLOYMENT

Transplant Surgery Research Fellow (Baltimore, MD; 07/11-present)

- The Johns Hopkins Hospital, Department of Surgery, Division of Transplant Surgery

Lecturer in Anatomy, Surgery, and Obstetrics/Gynecology (Kuala Lumpur, Malaysia, 09/13-10/13)

- Perdana University Graduate School of Medicine

Surgical First Assist Moonlighter (Randallstown, MD; 03/12-present)

- Northwest Hospital, Department of Surgery

Cardiac Surgery Intensive Care Moonlighter (Baltimore, MD; 07/11-present)

- The Johns Hopkins Hospital, Department of Surgery

Surgical Intensive Care Moonlighter (Baltimore, MD; 07/11-present)

- The Johns Hopkins Hospital, Department of Surgery

Plastic Surgery Moonlighter (Baltimore, MD; 10/11-present)

- The Johns Hopkins Hospital, Department of Plastic Surgery

Surgical Moonlighter (Baltimore, MD; 10/11-present)

- Mercy Medical Center, Department of Surgery

House Officer III (Baltimore, MD; 07/10-06/11)

- The Johns Hopkins Hospital, Department of Surgery

House Officer II (Baltimore, MD; 07/09-6/09)

- The Johns Hopkins Hospital, Department of Surgery

House Officer I (Baltimore, MD; 07/08-06/09)

- The Johns Hopkins Hospital, Department of Surgery

EDUCATION

Johns Hopkins University Bloomberg School of Public Health Graduate Training Program in Clinical Investigation (Baltimore, MD; 09/11-05/14)

- PhD in Clinical Investigation
- Expected completion: May, 2014

University of Michigan Medical School (Ann Arbor, MI; 08/02-05/08)

- Medical Doctorate Degree
- Graduation with Distinction in Research

University of Michigan School of Public Health, Department of Epidemiology (Ann Arbor, MI; 09/06-08/07)

- Master's of Science in Clinical Research

University of Michigan College of Literature, Science, & the Arts (Ann Arbor, MI; 09/98-12/01)

- Bachelor of Arts in Spanish and Biomedical Sciences
- Inteflex: Eight Year Combined BA/MD Program

Universidad de Salamanca (Salamanca, Spain; 06/00-08/00)

- Study Abroad Program

FELLOWSHIPS AND INTERNSHIPS

National Institutes of Health National Research Service Award (F-32) (Baltimore, MD; 01/12-06/13)

- Mentors: Drs. Dorry Segev and Robert Montgomery
- Division of Transplant Surgery, Johns Hopkins Department of Surgery

American Society of Transplantation/Astellas Clinical Science Fellowship Grant (Baltimore, MD; 07/11-01/12)

- Mentor: Dr. Dorry Segev and Robert Montgomery
- Division of Transplant Surgery, Johns Hopkins Department of Surgery

Johns Hopkins Clinical Research Scholars Postdoctoral Fellow KL-2 Award (Baltimore, MD; 07/11-01/12)

- Mentor: Dr. Dorry Segev and Robert Montgomery
- Division of Transplant Surgery, Johns Hopkins Department of Surgery

National Institutes of Health Clinical Research Training Program Fellow (Bethesda, MD; 08/07-06/08)

- Mentor: Dr. Bradford Wood
- Diagnostic Radiology Department, Section of Interventional Radiology, National Institutes of Health Clinical Center

Alpha Omega Alpha (AOA) National Medical Honor Society Carolyn L. Kuckein Student Research Fellow (Ann Arbor, MI; 04/07-06/08)

- Grant awarded for project entitled: *Effect of second-hand smoke on peripheral artery disease severity as measured by ankle-brachial index and patients' symptoms*

National Institutes of Health National Research Service Award (T-32) (Ann Arbor, MI; 08/06-08/07)

- Mentor: Dr. Gilbert Upchurch,
- Section of Vascular Surgery, University of Michigan Department of Surgery

Fulbright Fellow (Managua, Nicaragua; 08/05-05/06)

- Affiliated with La Universidad Nacional Autónoma de Nicaragua and El Centro de Investigaciones y Estudios de Salud (09/05-05/06)
- Primary Care Rotation at the Centro de Salud Sócrates Flores (Rehydration Unit for Patients with Diarrhea, Epidemiology, Obstetrics/Gynecology, General Outpatient Consults, Pediatrics) (10/05-12/05)
- Volunteer, National Rubella Vaccination Campaign (10/05-11/05)
- Spanish Language Instruction (10/05-03/06)
- Pediatric Burn/Reconstructive Surgery Rotation at Hospital Metropolitano/Asociación Pro Niños Quemados de Nicaragua (03/06-05/06)
- Thesis: Trabajadores Nicaragüenses y el Derecho a Confidencialidad Médica (Nicaraguan Workers and the Right to Medical Confidentiality)
- Smiletrain Cleft Palate/Lip Repair Mission (Quilalí, Nicaragua; 05/06)

William von Leibig/Harvard Research Fellow in Vascular Surgery (Boston, MA; 06/03-08/03)

- Mentor: Dr. Bruce Furie
- The Center for Thrombosis and Hemostasis at Beth Israel Deaconess Medical Center and Harvard Medical School
- Project: The role of the p-selectin molecule in arterial versus venous thrombosis

United States Department of State Intern (Santiago, Chile; 03/02-05/02)

- Consular Section, U.S. Embassy

Inteflex Primary Care Preceptorship Intern (Zeeland, MI; 05/99-06/99)

- Family Practice Associates

LEADERSHIP

Johns Hopkins Hospital Critical Care Committee (Baltimore, MD; 2012-2014)

- Committee Member

Johns Hopkins Physician Assistant Surgical Residency Advisory Committee

Baltimore, MD; 2010-2012)

- Committee Member

Johns Hopkins Hospital Cost of Radiology and Lab Tests Committee (Baltimore, MD; 2009-2011

- Committee Member

Johns Hopkins Surgery Residency Education/Program Review Committee

(Baltimore, MD; 2008-2011)

- Resident Representative

Johns Hopkins New Hospital Patient Care Model Committee (Baltimore, MD; 2008-2009)

- Resident Representative

University of Michigan Medical Affairs Advisory Committee (Ann Arbor, MI; 2006-2007)

- Graduate Student Representative

University of Michigan Student Relations Advisory Committee (Ann Arbor, MI; 2006-2007)

- Graduate Student Representative

University of Michigan Student Assembly (Ann Arbor, MI; 2006-2007)

- Medical School Representative

Medical School Curriculum Policy Committee (Ann Arbor, MI; 2002-2005)

- Class of 2006 Representative

Medical School Career Development Executive Committee (Ann Arbor, MI; 2002-2005)

- Student Representative

Association of American Medical Colleges Organization of Student Representatives

- Regional Delegate on Medical Education (04/02-04/03)
- Representative (2002-2005)

University of Michigan College of Literature, Science, & the Arts Student Government (Ann Arbor, MI; 1998-2001)

- President (2000-2001)

- Treasurer (1999-2000)
- Representative (1998-2000)

Vice President of Student Affairs Michigan Roundtable (Ann Arbor, MI; 1998-2001)

- Advisory Group Student Representative

HONORS AND AWARDS

- Young Investigator Travel Award, World Transplant Congress (07/14)
- Junior Investigator Award, American Society of Transplant Surgeons Winter Symposium (01/14)
- Top 14 Abstract Award, American Society of Transplant Surgeons Winter Symposium (01/14)
- The Johns Hopkins Department of Surgery Gershon Efron Award for Outstanding Clinical Research (06/13)
- Top 10 Abstract Award, American Society of Transplant Surgeons Winter Symposium (01/13)
- Top 10 Poster of Distinction Award, American Society of Transplant Surgeons Winter Symposium (01/13)
- Freedom to Recognize Commendation, Northwest Hospital (07/12)
- Poster of Distinction, American Transplant Congress (06/12)
- American College of Surgeons 2012 Advocacy Summit Travel Scholarship (03/12)
- Press Ganey Patient Satisfaction Survey Commendation, Johns Hopkins Bayview Medical Center (06/11)
- Travel Scholarship for 2010 Society for Vascular Surgery Annual Meeting (06/10)
- Johns Hopkins Hospital Shining Star Award for Service Excellence (05/10)
- Johns Hopkins University Department of Surgery Outstanding Surgical Intern Award (06/09)
- Johns Hopkins University Department of Surgery Intern Award for Communication and Collegiality with Nurses (05/09)
- Baltimore Academy of Surgery 2009 Resident Research Award (02/09)
- University of Michigan Department of Surgery 2008 Medical Student Research Award for Excellence in Surgery Research (04/08)
- University of Michigan Alumni Society Legacy Leadership Award Honorable Mention (03/07)
- Travel Scholarship for 2006 Society for Vascular Surgery Annual Meeting (06/06)
- Commendation for performance on Obstetrics/Gynecology Standardized Patient Interview (11/04)
- University of Michigan International Institute's "For a University of the World Essay Contest" winner (04/03)
- University Academic Honors (04/01, 12/01)
- Academic Class Honors (03/00)
- University of Michigan Regent's Merit Scholarship (09/98)

ARTICLES

- **Orandi BJ**, Garonzik-Wang JM, Massie AB, Zachary AA, Montgomery JR, Van Arendonk KJ, Stegall MD, Jordan SC, Oberholzer J, Dunn TB, Ratner LE, Kapur S, Pelletier RP, Roberts JP, Melcher ML, Singh P, Sudan DL, Posner MP, El-Amm JM, Shapiro R, Cooper M, Lipkowitz GS, Rees MA, Marsh CL, Sankari BR, Gerber DA, Nelson P, Wellen J, Bozorgzadeh A, Gaber AO, Montgomery RA, Segev DL. Quantifying the risk of incompatible kidney transplantation: A multi-center study. *Am J Transplant.* (in press)
- Van Arendonk KJ, Boyarsky BJ, **Orandi BJ**, James NT, Smith JM, Colombani PM, Segev DL. *Pediatrics.* 2014 (in press)
- Lonze BE, Zachary AA, Desai NM, Magro CM, **Orandi BJ**, Dagher NN, Singer AL, Carter-Monroe N, Nazarian SM, Segev DL, Streiff MB, Montgomery RA. Eculizumab prevents recurrence of antiphospholipid antibody syndrome and enables successful renal transplantation. *Am J Transplant.* 2014;14(2):459-65.
- Gupta N, Garonzik-Wang JM, Passarella RJ, Salter ML, Kucirka LM, **Orandi BJ**, Law AH, Segev DL. Assessment of resident and fellow knowledge of the organ donor referral process. *Clin Transplant.* (in press)
- Van Arendonk KJ, James NT, **Orandi BJ**, Garonzik-Wang JM, Smith JM, Colombani PM, Segev DL. Order of donor type in pediatric kidney transplant recipients requiring retransplantation. *Transplantation.* 2013;96(5):487-493.
- Van Arendonk KJ, **Orandi BJ**, James NT, Segev DL, Colombani PM. Living unrelated renal transplantation: A good match for the pediatric candidate? *J Pediatr Surg.* 2013;48(6):1277-1282.
- Van Arendonk KJ, James NT, Boyarsky BJ, Garonzik-Wang JM, **Orandi BJ**, Magee JC, Smith JM, Colombani PM, Segev DL. Age at graft loss after pediatric kidney transplantation: Exploring the high-risk age window. *Clin J Am Soc Nephrol.* 2013;8(6):1019-1026.
- Garonzik Wang JM, James NT, Van Arendonk KJ, Gupta N, **Orandi BJ**, Hall EC, Massie AB, Montgomery RA, Dagher NN, Singer AL, Cameron AM, Segev DL. The aggressive phenotype revisited: utilization of higher-risk liver allografts. *Am J Transplant.* 2013;13(4):936-942.
- Housri N, Coombs M, **Orandi BJ**, Pawlik TM, Koniaris LG. Ethics and the law: Is there common ground on informed consent for hospital outcome disparities? *Ann Int Med.* 2011;155:260-264.
- **Orandi BJ**, Blackburn S, Henke PK. Surgical mentors and mentees productivity from 1993-2006. *Am J Surg.* 2011;201:260-265.

- Sharma KV, Dreher MR, Tang Y, Pritchard W, Chiesa OA, Karanian J, Peregoy J, **Orandi B**, Woods D, Donahue D, Esparza J, Jones G, Willis SL, Lewis AL, Wood BJ. Development of “imageable” beads for transcatheter embolotherapy. *J Vasc Interv Radiol*. 2010;21(6):865-76.
- **Orandi B**, Eckhauser F. New anticoagulation strategies in patients with cancer and venous thromboembolism. *Am J Hematol Oncol*. 2010;9(2):65-66.
- **Orandi B**, Eckhauser F. Neoadjuvant chemoradiation therapy (CRT) improves disease-free survival (DFS) in patients with stage II or III rectal cancer. *Am J Hematol Oncol*. 2010;9(2):71-72.
- **Orandi BJ**, Dimick JB, Deeb GM, Patel HJ, Upchurch GR. A population-based analysis of endovascular versus open thoracic aortic aneurysm repair. *J Vasc Surg*. 2009;49:1112-6.
- **Orandi BJ**. The escalating importance of clinical research. *Virtual Mentor*. 2009;11(4):277-278.
- Linguraru MG, **Orandi BJ**, Van Uitert RL, Mukherjee N, Summers RM, Gladwin MT, Machado RF, Wood BJ: CT and image processing non-invasive indicators of sickle cell secondary pulmonary hypertension. In: Proceedings of 30th IEEE Engineering in Medicine and Biology Society (EMBS) Annual International Conference (2008) 859-862.
- Linguraru MG, **Orandi BJ**, Summers RM, Gladwin MT, Machado R F, Wood BJ.: Non-invasive quantification of sickle cell secondary pulmonary hypertension from CT image analysis. In: Proceeding of Radiological Society of North America (RSNA) Annual Meeting (2008) 98-99.
- **Orandi BJ**. *Being responsive to the world*. The Journal of the International Institute [The University of Michigan]. 2003 (Spring/Summer):10(3).

CHAPTERS

- **Orandi BJ**, Freischlag JA, Malas M. Publishing a clinical trial. In: Pawlik TM, Sosa JA (eds.), *Success in Academic Surgery: Clinical Trials*. London: Springer, 2014.
- **Orandi BJ**, Black JH. Open repair of abdominal aortic aneurysms. In: Cameron JL, Cameron AM (eds.), *Current Surgical Therapy*, 11th ed. Philadelphia, PA: Elsevier Saunders, 2013.
- **Orandi BJ**. Authored/edited multiple chapters. In: Meguid RA, Van Arendonk KJ, Lipsett PA (eds.), *The Johns Hopkins ABSITE Review Manual*, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2012.

- **Orandi BJ**, Black JH. Complications of endovascular aneurysm repair. In: Kpodonu J, Bonan R (eds.), *Endovascular and Hybrid Therapies for Structural Heart and Aortic Disease*, 1st ed. West Sussex, UK: Wiley-Blackwell, 2013.
- **Orandi BJ**, Black JH. Acute mesenteric ischemia. In: Dimick JB, Upchurch GR, Sonnenday C (eds.), *Clinical Scenarios in General Surgery*, 1st ed Philadelphia, PA: Lippincott Williams & Wilkins, 2012.
- **Orandi BJ**, Winter JM, Segev DL, Makary MA. Frailty and surgery in the elderly. In: Rosenthal RA, Zenilman ME, Katlic MR (eds.), *Principles and Practice of Geriatric Surgery*, 2nd ed. New York City: Springer, 2011.
- **Orandi BJ**. Know what to look for when patients with post-ERCP complications present to the emergency department. In: Mattu A, Chanmugam AS, Swadron SP, Tibbles CD, Woolridge DP (eds.), *Avoiding Common Errors in the Emergency Department*. Philadelphia: Lippincott Williams & Wilkins, 2010.
- Upchurch GR Jr, **Orandi BJ**, Eliason JL, Patel HJ, Williams DM, Rectenwald JE, Criado E, Deeb GM. Population-based analysis of endovascular versus open repair of thoracic aortic aneurysms. In: Yao JST, Pearce W, Matsamura J, Morasch M, Eskadari M (eds.), *Modern Trends in Vascular Surgery: Surgery of the Aorta and Its Body Branches*. People's Medical Publishing House, Shelton, CT, 2009.
- Upchurch GR Jr, **Orandi BJ**, Eliason JL, Patel HJ, Williams DM, Rectenwald JE, Criado E, Deeb GM. Population-based analysis of endovascular versus open repair of thoracic aortic aneurysms. In: Matsumura JS, Morasch MD, Pearce WH, Yao JST (eds.), *Techniques and Outcomes in Endovascular Surgery*. Greenwood Academic, Evanston, IL, pp 196-205, 2009.
- Linguraru MG, **Orandi BJ**, Mukherjee N, Van Uitert RL, Summers RM, Gladwin MT, Machado RF, Wood BJ: Semi-automated non-invasive CT assessment of sickle cell secondary pulmonary hypertension. In: *Workshop on Vasculopathy in Sickle Cell Disease (2008)* 197-198 (Science Award, extended abstract).

PRESENTATIONS

- **Orandi BJ**, Kucirka LM, Avery RK, Montgomery RA, Segev DL. Post-transplant infections in HLA-incompatible kidney transplantation: A multi-center study. Oral presentation. World Transplant Congress. San Francisco, CA. July 29, 2014
- **Orandi BJ**, Kucirka LM, Montgomery RA, Segev DL. National estimates and outcomes of incompatible live donor kidney transplantation amongst Medicare beneficiaries. Oral presentation. World Transplant Congress. San Francisco, CA. July 29, 2014

- **Orandi BJ**, Garonzik-Wang JM, Massie AB, Zachary AA, Montgomery JR, Van Arendonk KJ, Stegall MD, Jordan SC, Oberholzer J, Dunn TB, Ratner LE, Kapur S, Pelletier RP, Roberts JP, Melcher ML, Singh P, Sudan DL, Posner MP, El-Amm JM, Shapiro R, Cooper M, Lipkowitz GS, Rees MA, Marsh CL, Sankari BR, Gerber DA, Nelson P, Wellen J, Bozorgzadeh A, Gaber AO, Montgomery RA, Segev DL. Incompatible kidney transplantation risk and its relationship to CMS regulation: A multi-center study. Oral presentation. World Transplant Congress. San Francisco, CA. July 29, 2014
- **Orandi BJ**, Kraus ES, Alachkar N, Wickliffe C, Bagnasco SM, Montgomery RA, Segev DL. Outcomes of C4d-negative antibody-mediated rejection after kidney transplantation. Oral presentation. World Transplant Congress. San Francisco, CA. July 28, 2014
- **Orandi BJ**, Kucirka LM, Kumar K, Garonzik-Wang JM, Van Arendonk KJ, Montgomery RA, Segev DL. Prevalence of mental health disorders in HLA-incompatible transplant recipients vs. compatible recipients: A multi-center study. Poster presentation. World Transplant Congress. San Francisco, CA. July 27, 2014
- **Orandi BJ**, Kraus ES, Bagnasco SM, Van Arendonk KJ, Garonzik-Wang JM, Montgomery RA, Segev DL. Outcomes of concurrent cellular rejection in patients with antibody-mediated rejection. Poster presentation. World Transplant Congress. San Francisco, CA. July 27, 2014
- **Orandi BJ**, Kraus ES, Bagnasco SM, Van Arendonk KJ, Garonzik-Wang JM, Wickliffe C, Montgomery RA, Segev DL. Epidemiology and long-term outcomes of T-cell and antibody-mediated rejection on renal allografts. Oral presentation. European Renal Association-European Dialysis and Transplantation Association Congress. Amsterdam, Netherlands. June 3, 2014.
- **Orandi BJ**, James NT, Montgomery RA, Desai NM, Segev DL. Heterogeneity across transplant centers in the development of delayed graft function following deceased donor kidney transplantation. Poster presentation. European Renal Association-European Dialysis and Transplantation Association Congress. Amsterdam, Netherlands. June 1, 2014.
- **Orandi BJ**, Selvarajah S, Glebova N, Abularrage CJ, Black JH, Freischlag JA, Lum Y. Patients ≥ 40 Years With Neurogenic Thoracic Outlet Syndrome Have Similar Immediate Post-Operative Experiences As Patients < 40 Years. Oral presentation. Charing Cross Vascular Symposium. London, UK. April 5, 2014.
- **Orandi BJ**, Garonzik-Wang JM, Van Arendonk KJ, King EA, Gupta N, Montgomery RA, Segev DL. Sequelae of concurrent antibody- and cell-mediated rejection following kidney transplantation. Oral presentation. American Surgical Congress. San Diego, CA. February 6, 2014.

- **Orandi BJ**, James NT, Hall EC, Van Arendonk KJ, Garonzik-Wang JM, Gupta N, Desai NM, Segev DL. Variability of delayed graft function after deceased donor kidney transplantation across U.S. transplant centers. Quickshot oral presentation. American Surgical Congress. San Diego, CA. February 6, 2014.

- **Orandi BJ**, Selvarajah S, Mathioudakis N, Sherman RE, Arnaoutakis DJ, Haider AH, Black JH, Abularrage CJ. Disparities in major amputation rates between diabetics and non-diabetics with foot and ankle ulcers in the United States. Quickshot oral presentation. American Surgical Congress. San Diego, CA. February 4, 2014.

- **Orandi BJ**, Garonzik-Wang JM, Massie AB, Zachary AA, Montgomery JR, Van Arendonk KJ, Stegall MD, Jordan SC, Oberholzer J, Dunn TB, Ratner LE, Kapur S, Pelletier RP, Roberts JP, Melcher ML, Singh P, Sudan DL, Posner MP, El-Amm JM, Shapiro R, Cooper M, Lipkowitz GS, Rees MA, Marsh CL, Sankari BR, Gerber DA, Nelson P, Wellen J, Bozorgzadeh A, Gaber AO, Montgomery RA, Segev DL. Quantifying the risk of incompatible kidney transplantation: A multi-center study. Oral presentation. American Society of Transplant Surgeons Winter Symposium. Miami, FL. January 26, 2014.

- **Orandi BJ**, Garonzik-Wang JM, Van Arendonk KJ, King EA, Gupta N, Montgomery RA, Segev DL. Sequelae of concurrent antibody- and cell-mediated rejection following kidney transplantation. Poster presentation. American Society of Transplant Surgeons Winter Symposium. Miami, FL. January 24, 2014.

- **Orandi BJ**. Kidney transplantation in the immunologically high-risk patient. Johns Hopkins Department of Surgery Research Seminar Series. Baltimore, MD. November 7, 2013.

- **Orandi BJ**, Van Arendonk KJ, Garonzik-Wang JM, Chow E, Montgomery RA, Segev DL. Early antibody mediated-rejection portends worse long-term renal allograft and patient survival. Johns Hopkins Department of Surgery 2013 Surgical Research Day. Baltimore, MD. June 12, 2013.

- **Orandi BJ**, Van Arendonk KJ, Garonzik-Wang JM, Chow E, Montgomery RA, Segev DL. Early antibody mediated-rejection portends worse long-term renal allograft and patient survival. 7th Annual Johns Hopkins Department of Surgery Research Poster Session. Baltimore, MD. February 21, 2013.

- **Orandi BJ**, Van Arendonk KJ, Garonzik-Wang JM, Chow E, Montgomery RA, Segev DL. Early antibody mediated-rejection portends worse long-term renal allograft and patient survival. Distinguished Faculty Poster Review Session Presentation. American Society of Transplantation Cutting Edge of Transplantation Meeting. Phoenix, AZ. February 15, 2013.

- **Orandi BJ**, Van Arendonk KJ, Garonzik-Wang JM, Chow E, Montgomery RA, Segev DL. Early antibody mediated-rejection portends worse long-term renal allograft and

patient survival. Oral presentation. American Society of Transplant Surgeons Winter Symposium. Miami, FL. February 3, 2013.

- **Orandi BJ**, James NT, Hall EC, Garonzik-Wang JM, Van Arendonk KJ, Gupta N, Segev DL. Transplant center associations on the development of delayed graft function following deceased donor kidney transplantation. Poster presentation. American Society of Transplant Surgeons Winter Symposium. Miami, FL. February 1, 2013.

- **Orandi BJ**, Garonzik-Wang JM, Gupta N, Van Arendonk KJ, Lonze BE, Zachary AA, Alachkar N, Kraus ES, Locke JE, Nazarian SM, Dagher NN, Desai NM, Segev DL, Montgomery RA. C5 complement inhibition as salvage therapy for severe antibody-mediated rejection following HLA-incompatible renal transplantation. Poster presentation. American Society of Transplant Surgeons Winter Symposium. Miami, FL. February 1, 2013.

- **Orandi BJ**, Garonzik-Wang JM, Gupta N, Van Arendonk KJ, Lonze BE, Zachary AA, Alachkar N, Kraus ES, Locke JE, Nazarian SM, Dagher NN, Desai NM, Segev DL, Montgomery RA. C5 complement inhibition as salvage therapy for severe antibody-mediated rejection following HLA-incompatible renal transplantation. Poster presentation. International Congress of The Transplantation Society. Berlin, Germany. July 19, 2012.

- **Orandi BJ**, James NT, Hall EC, Garonzik-Wang JM, Van Arendonk KJ, Gupta N, Segev DL. Transplant center associations on the development of delayed graft function following deceased donor kidney transplantation. Poster presentation. American Transplant Congress. Boston, MA. June 2, 2012.

- **Orandi BJ**. Contrast-induced nephropathy. Invited presentation. Maryland Regional Vascular Society. Annapolis, MD. May 14, 2011.

- **Orandi BJ**, Dimick JB, Deeb GM, Patel HJ, Upchurch GR. A population-based analysis of endovascular versus open thoracic aortic aneurysm repair. Oral presentation. Baltimore Academy of Surgery. Baltimore, MD. February 18, 2009.

- **Orandi BJ**, Blackburn SA, Henke PK. Surgical mentors and mentees productivity from 1993-2006. Oral presentation. Oral presentation. 4th Annual Academic Surgical Congress, Society of University Surgeons. Fort Myers, FL. February 5, 2009.

- **Orandi BJ**, Linguraru MG, Al-Bakir I, Summers RM, Gladwin MT, Machado RF, Wood BJ. Non-invasive quantification of sickle-cell-associated pulmonary hypertension from CT image analysis. Oral presentation. National Institutes of Health-Clinical Research Training Program Scientific Presentations. Bethesda, MD. May 29, 2008.

- **Orandi BJ**, Linguraru MG, Al-Bakir I, Summers RM, Gladwin MT, Machado RF, Wood BJ. Non-invasive quantification of sickle-cell-associated pulmonary hypertension from CT image analysis. Poster presentation. National Institutes of Health Research Festival. Bethesda, MD. May 8, 2008.

• **Orandi BJ**, Linguraru MG, Al-Bakir I, Summers RM, Gladwin MT, Machado RF, Wood BJ. Non-invasive quantification of sickle-cell-associated pulmonary hypertension from CT image analysis. Poster presentation. National Institutes of Health-Pfizer Research Exhibition. Groton, CT. May 2, 2008.

• **Orandi BJ**, Sharma V, Upchurch GR Jr. Perioperative outcomes after open and endovascular repair of intact thoracic aortic aneurysms: results of the Nationwide Inpatient Sample. Oral presentation. Society of Clinical Vascular Surgery. Las Vegas, NV. March 7, 2008.

• **Orandi BJ**, Sharma V, Upchurch GR Jr., Napolitano LM. Is there a “July phenomenon” in central venous catheter placement? Results of the Nationwide Inpatient Sample, 1998-2005. Poster discussion. Society of Critical Care Medicine. Honolulu, HI. February 4, 2008.

• **Orandi BJ**. Protecting the right to personal health information privacy in Nicaragua. International Health and Human Rights poster session. American Public Health Association. Washington, DC. November 5, 2007.

• **Orandi BJ**. VIH y SIDA (HIV and AIDS). Invited oral presentation. First Annual Forum on HIV/AIDS. Waslala, Nicaragua. December 1, 2005.

• **Orandi BJ**. In vivo imaging of arterial and venous thrombi formation. Oral presentation. William J. von Liebig Summer Research Program in Vascular Surgery Graduation. Boston, Massachusetts. August 20, 2003.

EXTRACURRICULAR ACTIVITIES/ADDITIONAL CERTIFICATIONS

- Advanced Trauma Life Support Certification (03/2014)
- Peer Reviewer, Archives of Surgery (2012-2013)
- Basic Life Support Certification (01/2012)
- Cardiovascular and Respiratory Section Editor. In: Meguid RA, Van Arendonk KJ, Lipsett PA (eds.), The Johns Hopkins ABSITE Review Manual, 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2012.
- Virtual Mentor: American Medical Association Journal of Ethics Theme Issue Editor (02/08-08/09)
- Volunteer Tutor for the Washtenaw Literacy Society (02/07-07/07)
- Volunteer, Migrant Farm Worker Clinic, Manchester, MI (06/06-10/06)
- Audited M4 elective, “Law, Medicine, and Society” (03/04)
- Medical Students for Cuba Humanitarian Mission (02/03)
- Server, Palio Italian Restaurant (10/99-05/02)

LANGUAGES

- Spanish (fluent)

- Farsi (reading and writing at a fifth-grade level)

PROFESSIONAL SOCIETY MEMBERSHIPS

- American Society of Transplantation
- Association for Academic Surgery
- American College of Surgeons Resident and Associate Society
- Fulbright Alumni Society
- University of Michigan Medical Center Alumni Society

BRIEF BIOSKETCH

Dr. Babak J. Orandi was born in St. Cloud, MN and was raised in Bloomfield Hills, MI. He attended the University of Michigan, where he was enrolled in the Inteflex Integrated Premedical/Medical Program. As an undergraduate student, he was elected president of the University of Michigan College of Literature, Science, & the Arts Student Government. He graduated in December, 2001 with a B.A. in Spanish. Prior to starting medical school in 2002, he worked as an intern for the U.S. Department of State at the U.S. Embassy in Santiago, Chile. During medical school, Dr. Orandi spent a summer as a William von Leibig/Harvard Research Fellow in Vascular Surgery. From 2005-2006, he lived and worked in Managua, Nicaragua under the auspices of a Fulbright Fellowship. He also completed a Masters in Clinical Research at the University of Michigan School of Public Health and spent a year at the National Institutes of Health as a Clinical Research Training Program Fellow before graduating from medical school with Distinction in Research. In 2008, he began his General Surgery residency at the Johns Hopkins Hospital. At the end of his first year, he was awarded the "Intern of the Year" award from the Department of Surgery. In 2011, he joined the Epidemiology Research Group for Organ Transplantation in the Division of Transplant Surgery, led by Dr. Dorry Segev. Dr. Orandi has an interest in incompatible kidney transplantation and health policy. In his free time, he enjoys running, traveling, reading, and yoga.